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Biomonitoring methods for assessing the impacts of nitrogen pollution: refinement and testing

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Executive Summary

- The conservation agencies are required to identify, monitor and protect sites designated for nature conservation under UK and European legislation. The ability to determine the impacts of enhanced N concentrations and N deposition is important for assessing effects on site condition and integrity. Currently, assessment of atmospheric pollution effects on these sites is not part of the common standards monitoring. The use of biomonitoring methods is one approach, which could provide an early warning of sites at risk from N deposition.

- This report describes a two-part field study, which applied bioindicator methods in parallel. Firstly methods were applied to 4 key 'intensive' UK sites with contrasting habitats and atmospheric N concentrations and deposition. Then secondly, bioindicator methods identified from the intensive study were applied at the UK scale (extensive study) at 32 sites with a range of habitat types, NH_3 concentrations and N deposition.

- The bioindicator methods tested at the intensive sites were a) chemical (foliar N content and soluble foliar ammonium concentration using pleurocarpous mosses), b) standardised grass bioindicator (*Deschampsia flexuosa*): effects on biomass and foliar N concentrations, c) Ellenberg N index and d) epiphytic lichen frequency and species composition, including associated measurement of bark pH. The methods selected for application at the UK scale were the chemical analysis of mosses (a) and epiphytic macrolichen frequency and community composition (d).

- The use of conservation and environment agencies' staff to record epiphytic macrolichen frequency and to sample lichens and pleurocarpous mosses for the UK extensive study was successful. The quality of sampling and recording was high and their participation enabled a much more comprehensive study to be undertaken, as well as dissemination of the methodology.

Ellenberg N Index

- Ellenberg N index was shown to be a useful bioindicator method at the intensive sites for assessing the N status along a known gradient of NH_3 concentration, NO_2 concentration and N deposition.

- At the sites dominated by long-range wet N deposition, the use of Ellenberg N index did not detect significant change in vegetation due to N deposition.

- The application of an acidophyte-nitrophyte index for vascular plants and bryophytes was tested at the intensive sites and could provide a more sensitive measure of N deposition and eutrophication impacts to a target habitat.

Standardised grass transplants

- The grass, *Deschampsia flexuosa* was found to be a robust bioindicator of NH_3, NO_2 concentrations and N deposition at sites with a large gradient of atmospheric N with the nitrogen impacting strongly on above ground biomass, foliar N contents and soluble ammonium concentrations. However, *Deschampsia flexuosa* exposed for 3 months at

the sites dominated by wet N deposition did not show any changes in biomass or foliar N concentrations in relation to amount of N deposition.

Chemical methods (foliar N content and soluble ammonium concentration)

- Both foliar N content and soluble ammonium concentration proved to be robust bioindicator methods for the detection of N impacts when using *Deschampsia flexuosa* and the pleurocarpous mosses at sites with a strong gradient in NH_3, NO_2 concentration and N deposition.

- Overall, the UK extensive study showed a positive but weak correlation between moss foliar N content, soluble ammonium concentrations and NH_3 concentration and N deposition. The low overall relationship indicates that (for the amount of sample replication used here) for sites with diffuse sources of N deposition over a modest range, the foliar N concentration and soluble ammonium concentration of bryophytes may not provide a sufficient signal for spatial comparisons at one time. Increased replication of samples from wider search areas is therefore recommended for studies at such low N deposition levels.

- There was strong interspecies variation in the sampled mosses in response to NH_3 and N deposition. In general the response to NH_3 concentration was greater than that found for N deposition. This could be due to a number of habitat and climatic factors including interactions with regional precipitation differences.

Lichens diversity

- Frequency of lichen indicator species was found to be a robust bioindicator method at both the 4 key intensive sites and at the UK scale. The main restriction on the method is the requirement for the presence of deciduous trees at the sampling site. Comparison of lichens growing on twigs and trunks showed that those on twigs were more sensitive to NH_3 concentration. This is associated with the higher bark pH of twigs.

- Macrolichen frequency was recorded in the UK extensive study, which found nitrophyte lichen species increased on twigs and trunks with increasing atmospheric NH_3 concentration. The loss of acidophyte lichen species was found to occur at lower ammonia concentrations than the subsequent increase in nitrophyte species.

- Comparison of expert and non-expert lichenologists sampling and field identification of epiphytic lichens showed that a simplified recording system using frequency of macrolichens could be used to detect change in site condition resulting from N deposition impacts. At the UK scale, the lichens were found to respond most closely to the NH_3 concentration.

Application of the results

- The results are used to indicate the potential for significant adverse effects at the different UK intensive sites and extensive sites. In many cases the results of different biomonitoring methods confirm a wider picture of effects or no effects at the sites. Hence increased robustness in the application of nitrogen bioindicators and biomonitoring may be obtained by using several approaches simultaneously.

- The concept of robustness may be extended by considering different nitrogen indicators in a “biomonitoring chain” from source to conservation relevant impact: emission, air

concentration, deposition, N accumulation, physiological response, injury, growth response, species composition change (most sensitive species), species composition change (designated species for conservation).

- While it is difficult to measure all of these stages, selecting the easier methods from along the range of this biomonitoring chain, both increases robustness (multiple methods), and makes the link between source attribution (methods closest to emission) and adverse effect (methods closest to impact on designated species).

Technical Summary

Background and structure of the report

1. The conservation agencies are responsible for the identification and protection of designated sites of nature conservation (such as Sites of Special Scientific Interest (SSSIs), Special Areas of Conservation (SACs) and Special Protection Areas (SPAs). An assessment of these designated sites is carried out on a 6-year cycle to monitor the condition of the designated interest feature/features for that specific site, with key attributes being identified and targets set for each feature.

2. Although there is concern about the potential impacts of atmospheric nitrogen (N) deposition on conservation areas with N sensitive plant species, an assessment of N impacts is currently not explicitly included in the Common Standards Monitoring (CSM) of designated sites. An assessment of air pollution impacts, including N, is also required as part of the permitting process for the Pollution Prevention and Control Regulations and to fulfil the obligations placed on competent authorities, such as Scottish Environmental Protection Agency (SEPA), by the Habitats Regulations.

3. A comprehensive review of existing biomonitoring methods for determining the impacts of N deposition on plant species and habitats was produced by Sutton *et al*. (2004a) on behalf of the Joint Nature Conservation Committee (JNCC, Report No: 356). That report reviewed N biomonitoring approaches and identified robust N bioindicator methods, which could be applied by the conservation agencies to designated nature conservation areas to assess potential N impacts. The study also included a field component at an agricultural NH_3 point source, where several N biomonitor techniques were examined in parallel.

4. This report describes the work commissioned by JNCC and Scotland and Northern Ireland Forum for Environmental Research (SNIFFER) to assess rigorously a short-list of specific N biomonitoring methods identified by Sutton *et al*. (2004a) for wider application by the conservation agencies and SEPA and EHS at designated nature conservation sites for pollution impact assessment.

5. The present report is structured in six parts:

   a) Intensive site study.

      - This study assesses in detail the simplified biomonitoring methods (Ellenberg N index, Lichen diversity and the chemical methods: foliar N and soluble ammonium concentration in mosses) tested in parallel at 4 key sites with contrasting N sources and habitat types.

b) Extensive UK study.

- This study evaluates the selected simplified N bioindicator methods identified in the intensive site study at the UK scale. The methods applied were the chemical methods (foliar N content and soluble ammonium concentration) and the lichen diversity (with associated measurement of bark pH). The study also compares expert and non-expert epiphytic lichen diversity identification and sampling quality at a small number of UK extensive study sites.

c) Synthesis of the tested biomonitoring methods.

- Synthesis of the intensive and UK scale extensive site biomonitoring method results.
- Evaluation of the robustness, applicability of the bioindicator methods for use by the conservation and environment agencies.

d) Appendix I summarizes the development of an improved methodology for the chemical biomonitor method, soluble plant NH_4-N concentration.

e) Appendix II summarizes a pilot study comparing the effects of NH_3 along a concentration gradient on the above and below ground biomass and foliar N concentrations of the standardised grass biomonitor (*Lolium multiflorum).*

f) Appendix III details the lichen diversity sampling protocols and site description data sheets used by the conservation and environment agencies' staff in sampling for the extensive UK study.

Method development and testing at four key intensively measured sites.

6. The sites were selected for a contrasting range of habitat and atmospheric N deposition and N form.

a) **A lowland mixed deciduous woodland.** N source: agricultural NH_3 point source (poultry farm adjacent to Piddles Wood SSSI, Dorset).

b) **A lowland mixed deciduous and conifer woodland.** N source: vehicle emissions (NO_x) from the M74 motorway at a site near Happendon, Lanarkshire, Scotland.

c) **An upland and lowland moorland.** N source: wet deposition in precipitation (NH_x and NO_y) comparing Auchencorth Moss and Bowbeat Hill, sites in the southern uplands of Scotland.

d) **An N manipulation study on blanket bog vegetation**. N sources: dry NH_3 and the two main N species in precipitation (NO_3^- and NH_4^+) at the CEH Edinburgh Whim Moss experimental facility.

Application of the Ellenberg indicator approach at the four intensive sites

7. Ellenberg devised a comprehensive indicator system for vascular plants of central Europe (Ellenberg 1979; Ellenberg *et al.* 1992) to describe the response of individual species to a range of ecological conditions (light, temperature, continentality, moisture, pH and N). The Ellenberg N index is a robust indicator of enhanced N deposition, which has been used extensively in Europe to indicate vegetation change due to increased atmospheric N deposition.

8. The Ellenberg N index method relies on the preclassification of different species preferences to N availability, which were modified by Hill *et al*. (1999) for British conditions. Although the method is relatively simple, it requires a sound botanical knowledge to identify accurately a wide range of species.

9. In the current study, the Ellenberg N Index was tested at all the intensive study sites, but excluded (due to resource availability) from the N manipulation study at Whim Moss.

10. Ellenberg N Index was particularly useful in assessing the N status along known gradients in N deposition, confirming the strength of the method in indicating enhanced N deposition, and providing an important standard for comparison between sites, and within sites on a spatial and temporal scale. The determination of the Ellenberg N index along a gradient of NH_3 concentration and N deposition at Piddles Wood, and a gradient of NO_2 concentrations and N deposition at Happendon Wood showed changes in vegetation composition, which could be attributed to N deposition.

11. Ellenberg N Index appears to be a weaker predictor of the relative N status of sites dominated by wet deposited N. At the two moorland sites both of naturally low N status, Auchencorth Moss and Bowbeat Hill, but with different atmospheric N inputs, mean Ellenberg N Index did not indicate any N driven change in species composition. At such sites, the presence of mainly stress-tolerant, low N value species and the absence of propagules of high Ellenberg Index plants may restrict changes in the mean Ellenberg N Index.

12. The first attempt to apply an index based on the selection of acidophyte and nitrophyte species (vascular + bryophytes) provided useful information on the eutrophication of the sites. In this approach, previously applied only for lichens, the assessment is based not on the full species list at a site, but only on those species known to favour nitrogen-rich or nitrogen-poor N conditions.

13. The acidophyte-nitrophyte approach for higher plants, bryophytes and lichens has the potential to provide a more sensitive measure of N deposition induced changes, than the Ellenberg approach, since it focuses on the species changes most relevant for each habitat. For example, at Piddles Wood the flora was shown to be dominated by nitrophyte species at NH_3 concentrations greater than 3 $\mu g\ m^{-3}$. There is now a need to develop the database of key species of acidophytes and nitrophytes for major habitats, followed by site evaluation in the UK to develop the robustness and scope of this approach.

14. The application of both the Ellenberg and acidophyte-nitrophyte methods by conservation and environment agencies staff requires training in botanical identification. However, while a full botanical survey is necessary to obtain an accurate Ellenberg Index, the simpler index based on the acidophyte/nitrophyte balance may require less training (as fewer species need to be identified). Development of key acidophyte and nitrophyte species for habitat types should facilitate application by conservation officers.

Application of *D. flexuosa* as a standard biomonitor at the intensive sites.

15. *Lolium multiflorum* has been used extensively as a standardised grass biomonitor in the past and was tested here in relation to N deposition. It can be used to assess N deposition along an exposure gradient with short exposure periods of 20-50 days.

However, in areas with diffuse N inputs from long-range transport (i.e. no strong individual local source), and low N deposition the exposure period required is much longer (60 + days), mainly due to the episodicity of precipitation and wet deposition. The fast growing *Lolium* spp. is therefore unsuitable and a slower growing grass species is required. This study investigated whether *Deschampsia flexuosa* could be used as an alternative to the faster growing *Lolium* spp.

16. The standardised grass transplant system with a reservoir of water worked well in all the different habitats and environmental conditions. The method was relatively cheap to use and does not require specialist equipment (the equipment cost per six plant tray was ~ £25). There would be an analytical cost of ~ £20 per sample for tissue N content and soluble NH_4-N concentration. The grass N biomonitors only required minimal management over the three months exposure period with a site visit every 10-14 days.

17. The measurements show that *D. flexuosa* could be used effectively as a grass N biomonitor especially at sites with a local point source, such as intensive agricultural livestock units. However, the application of standardised grass N biomonitors at sites with diffuse wet N inputs from long-range pollutant transport and lower N deposition values is not an effective method to monitor N deposition over a short period (0-3 months comparison between Bowbeat Hill and Auchencorth Moss). The germination and propagation of the *D. flexuosa* seed was not as straight forward as for *Lolium* spp. Therefore, further work to improve the germination rate/propagation techniques for this species is required.

18. Significant increases in above ground biomass, tissue N content and soluble NH_4-N concentration were found with increasing NH_3 concentration and N deposition in *D. flexuosa* plants after 3 months exposure at the intensive agricultural site at Piddles Wood.

19. Contrary to the results adjacent to the poultry unit, the biomass of the *D. flexuosa* grass biomonitors decreased with increasing NO_2, NH_3 concentrations and N deposition at Happendon Wood, the site adjacent to the M74 motorway. This decrease in biomass suggests that factors other than simple N supply (which would have a positive effect) associated with vehicle emissions have a negative impact on *D. flexuosa* growth (e.g. NO_2 toxicity, particle emissions, road salt etc). There were strong linear (negative) relationships with log NO_2 concentration and log N deposition and tissue N content but not with soluble NH_4-N concentrations. This would indicate that for sites with high NO_2 concentrations the soluble NH_4-N concentration method is less influenced by the NO_2 and other transport related pollutants.

20. No effects on grass bioindicator plant biomass and foliar chemistry were found for the sites with contrasting wet N deposition, Auchencorth Moss and Bowbeat Hill. This indicates that an exposure period of 3 months at sites with atmospheric N deposition derived principally from NO_3^- and NH_4^+ in precipitation is insufficient to detect impacts on grass biomonitors. The interacting effects of altitude, temperature and low N deposition appear to be responsible for the lack of response found. Further work is required to test grass biomonitors at long-range sites over a longer exposure period (6-12 months) to determine if N deposition can be detected in the grass foliage.

21. At the experimental manipulation site (Whim Moss) comparing wet and dry N deposition the mean tissue N content and soluble NH_4-N concentration were higher but not significantly in the NH_4-N treatments compared to the NO_3-N treatments. However, the biomass was greater in the NO_3-N treatments at both 32 and 64 kg N ha^{-1} y^{-1} and this is reflected in the higher N inventory. These different results point to the potential for

the grass biomonitor approach to distinguish the impacts of oxidised versus reduced N deposition. All N treatments significantly increased foliar N inventory compared to the control.

22. Comparison of wet and dry NH_x treatments (3 month exposure period at Whim Moss) show that at 10 kg N ha^{-1} y^{-1} inputs (estimated N deposition for 3 month experimental period for 64 kg N ha^{-1} y^{-1} treatments) the *D. flexuosa* biomass, foliar N concentrations and N inventory were all increased in wet treatment NH_4-N compared to the equivalent dry NH_3 treatment.

Application of tissue and soluble nitrogen determination in mosses as bioindicators at the intensive sites.

23. Total tissue N content is a widely recognised biomonitor of N impacts in a range of vegetation species. The use of soluble NH_4-N concentration of foliage has been measured in several previous studies, with much more recent application of this method as an N bioindicator (Sutton *et al*. 2004a). In the current study, the method has been compared directly with total tissue N and both have also been compared directly at both the intensive and UK scale extensive sites.

24. There were strong, robust relationships between tissue N content, soluble NH_4-N concentration in the mosses *Eurhynchium praelongum* and *Eurhynchium striatum* with NH_3 concentration and N deposition at the agricultural NH_3 source site (Piddles Wood). This indicates that both these chemical methods can be used effectively as bioindicators of N impacts at designated sites with a strong local N point source. There were differences between the two species with *Eurhynchium praelongum* appearing to be N saturated at the high NH_3 concentrations/N deposition. As both species were found within 5 m of the poultry house this would indicate that the two *Eurhynchium* species have a high tolerance to N.

25. The response to N deposition was much larger for soluble NH_4-N concentration than it was for tissue N content. Overall tissue N content increased by a factor of 2.7 in both *Eurhynchium praelongum* and *Eurhynchium striatum* while the soluble NH_4-N concentration increased by a factor of 20 for *Eurhynchium praelongum* and by a factor of 40 for *Eurhynchium striatum.*

26. Overall, the N content and soluble NH_4-N concentrations in the four moss species decreased with distance from the M74 motorway site (Happendon Wood). There was a relatively poor relationship between tissue N content, soluble NH_4-N concentration and NH_3 concentration (R^2=0.23 and 0.43 respectively). As the NH_3 concentrations measured were low along the gradient away from the M74 (1.27–0.45 µg m^{-3}), this suggests that other factors (including NO_2 concentrations) could be influencing N uptake as strong linear relationships were found between soluble NH_4-N concentration and both log NO_2 concentration and log N deposition. Although, a weak relationship was found between tissue N content of the four moss species and log N deposition (R^2=0.26) there was a much stronger relationship with log NO_2 concentration (R^2=0.96). The results show that the two chemical methods differ in their response to the atmospheric pollutants at Happendon Wood. This indicates that the application of individual chemical methods must be tailored to the pollutant at the site and also highlights the need for further research to determine what chemical method is applicable for different atmospheric N pollutants.

27. The results for the sites dominated by long-range transport of wet N deposition, Auchencorth Moss and Bowbeat Hill, suggest that the use of the foliar N bioindicator

methods would only be applicable with long-term monitoring as an 'early warning' of N deposition increases.

28. There were strong positive log-linear relationships (in *H. jutlandicum*) between tissue N content (% dry weight) and soluble NH_4-N concentration and NH_3 concentration along the NH_3 gradient at Whim Moss. The mean monthly NH_3 concentration ranged from 0.5 $\mu g\ m^{-3}$ to 70 $\mu g\ m^{-3}$ along the transect. The results show that both chemical methods were able to detect differences in N in *H. jutlandicum* after 2 years exposure to NH_3 concentration. The magnitude of responses was broadly proportional to the log of NH_3 concentration.

In the wet N deposition treatments at Whim Moss:

29. Differences were found between N forms in the wet N treatments. There was a strong linear-log relationship between tissue N content (% dry weight) and soluble NH_4-N concentration and wet N deposition of both N forms in *H. jutlandicum*. However, the increases in foliar N per unit N were greater in the NH_4-N treatments than the NO_3-N treatments.

30. The tissue N content (% dry weight) increased significantly when compared to ambient N deposition (10 kg N $ha^{-1}\ y^{-1}$) in both forms of N at N deposition of 32 and 64 kg N $ha^{-1}\ y^{-1}$.

31. In contrast, soluble NH_4-N concentrations were only significantly increased in the NH_4Cl, 64 kg N $ha^{-1}\ y^{-1}$ treatment compared to the control and the other NH_4-N and NO_3 –N treatments. This result suggests that a critical threshold wet deposition may have been exceeded in this treatment for soluble NH_4-N concentrations.

32. The comparison between dry NH_3, wet NO_3-N and wet NH_4-N inputs shows that these forms of N input do not all have the same impact on foliar N and soluble NH_4-N concentrations of mosses. For tissue N the sensitivity appears to be highest for NH_3, intermediate for wet NH_4-N and lowest for wet NO_3-N. This differentiation is similar but even stronger for soluble NH_4-N in mosses, which was very sensitive to NH_3, but only responded to high levels of wet N inputs.

Application of lichens diversity methods as N bioindicators at the intensive sites.

33. Transects were undertaken at Piddles Wood adjacent to a poultry unit with a NH_3 point source, and adjacent to the M74 motorway at Happendon Wood. At other sites (Whim Moss and Auchencorth Moss and Dunslair Hill near Bowbeat Hill) a basic comparison was made due to the limited availability of suitable trees). At these sites all lichen species were investigated on trunks and twigs, allowing "nitrophyte" and "acidophyte"species values to be calculated based on a modification of the van Herk approach. Ellenberg Index values were used as described in Wirth (1992).

34. The results showed that epiphytic lichen communities of twigs are strongly correlated with NH_3 concentrations. Acidophyte lichens on twigs are more sensitive to NH_3 than those on trunks, associated with higher bark pH of twigs than trunks. In addition lichen communities of trunks may carry relict species from previous conditions, allowing assessment of recent changes in NH_3 exposure based on a comparison of acidophyte and nitrophyte communities on twigs and trunks.

35. Lichen on trunks may also cover a wide tree age range and be subject to variation in environmental conditions such as shade. Where the same trees species could be used throughout the transect, as at Piddles Wood, the results showed a good correlation with ammonia concentrations. By contrast, in cases where tree species and habitat homogeneity varied, as across the M74 transect at Happendon Wood, the results were more difficult to interpret. The most consistent results were in the loss of acidophytes on acid-barked tree species at relatively low levels of NH_3 in all sites suggesting that the effects of ammonia on areas of natural vegetation are more widespread than previously thought. In sites where acidophyte vegetation was naturally dominant, loss of acidophytes was more conspicuous than the appearance of nitrophytes, these being often slow to colonise.

36. The results also demonstrated that in local situations on the same tree species that bark pH was highly correlated with atmospheric NH_3 concentrations.

Comparison of expert and non-expert lichen sampling.

37. In its original application, the method of van Herk (1999) provided a complex sampling method recording the presence of both macrolichens (foliose and fruticose species) and crustose lichens. While this method was previously shown in the UK to give good results (Sutton *et al*. 2004a), the scoring system was labour intensive and it required advanced lichen identification skills. The results of testing in a range of conditions; parkland at Bush Estate in Midlothian, coastal woodland at Stackpole, Pembrokeshire, an upland site at Pwll Peiran, Ceredigion and an inland oak wood at Yarner Wood in S. Devon demonstrated that the simplified recording system used in this study, based on macrolichen frequency forms a reliable basis to detect responses to increased NH_3 and N deposition.

38. The increase in nitrophytes on twigs at lower NH_3 concentrations than their appearance on trunks is consistent with the higher pH on twigs and the results of the extensive survey. There was also less difference in the results from macrolichen and total species sampling on twigs than on trunks where crustose species may be dominant. Following the testing of epiphytic macrolichen indicator species against pollution and environmental data the use of a standardised method and illustrated guide to indicator species would permit their widespread use in the UK.

Synthesis of bioindicator and biomonitoring results from the four intensive sites.

39. The comparison of the methods at Piddles Wood (agricultural NH_3 point source) show that all the simplified boimonitoring methods tested were found to be robust and could be applied by the conservation agencies at sites where there is a defined point source and a strong gradient of N deposition/concentrations.

40. The results for the other intensive site with a defined source, the M74 motorway at Happendon Wood, showed that Ellenberg index was suitable, the soluble NH_4-N concentration method was robust for mosses and *D. flexuosa*, but the other methods were considered to be suitable but only under specific conditions i.e. larger N concentration gradients.

41. None of the biomonitoring methods tested at the diffuse N source wet N dominated sites (Auchencorth Moss or Bowbeat Hill) were sufficient to show a statistically significant increase with N deposition. While soluble foliar ammonium of bryophytes increased as expected, tissue N actually decreased, possibly due to a lower ratio of dry to wet deposition at the high deposition site (Bowbeat Hill).

42. For higher plants and bryophytes, the Ellenberg N Index and a new modified acidophyte/nitrophyte index for these plants provide measures of current species composition and the extent to which nitrophyte species dominate a site. They confirm the current status of the site within the NVC classification and can show areas where change has already occurred. With comprehensive botanical knowledge, the Ellenberg index is straightforward to apply and has the added benefit of providing a species list for the site. The new acidophyte/nitrophyte index and future refinement of this approach using key species is simpler to apply with limited botanical training, as well as being more sensitive to the key species responses to N.

43. Foliar N chemistry measurements are a more sensitive indication of N exposure than species changes for higher plants and show the potential for change and damage to the 'health' of the habitat. These methods can act as an 'early warning' of potential N impacts to a designated site These techniques also provide a robust approach, which can be conducted cost-effectively in one-off surveys of spatial differences or as part of long-term monitoring programmes.

44. Use of standardised grass plants as biomonitors of N deposition has the advantage that the effects may be demonstrated visually over the short-term through altered biomass, as well as in foliar N concentrations and the above ground plant N inventory. For studies on diffuse sources of N deposition, such as enhanced wet deposition, a longer time period (several months) is necessary both to integrate the atmospheric inputs and to detect a significant response.

45. The comparison shows that while the different methods give broadly the same result, some are more sensitive than others, or respond differentially to different forms of atmospheric N supply. In simple terms, robustness in biomonitoring for N may be found by using several different approaches.

Testing of nitrogen bioindicator methods on the UK scale.

46. The chemical methods (tissue N content and soluble NH_4-N concentration) in pleurocarpous mosses and the lichen diversity methods were selected for use in the extensive UK scale study (32 sites throughout the UK, selected for a range of habitat types and atmospheric pollutant inputs). The use of Ellenberg N index for higher plants and standardised grass biomonitors (intensive study) were not practical within the confines of the study for wider application at the UK scale.

47. Local conservation agency staff and SEPA, CEH and NHM staff participated in the moss sampling, the lichen diversity measurements and collection of bark samples for pH measurements on the trunks and twigs. If required, local officers were given basic training (including a short training course) in moss and lichen identification and instruction on the application of the sampling protocols. All sites were provided with a sampling pack, which included sampling protocols and a ladder quadrat for the lichen survey of the tree trunks.

48. The criteria for site selection was based on a) availability of atmospheric monitoring at the site, b) the site being of conservation interest and c) the availability of local conservation/environment agencies' staff to conduct the lichen survey and vegetation sampling. The UK sites selected were all sites dominated by diffuse N deposition, with approximately 44% dominated by dry N deposition and the 54% by wet N deposition. A

site was assessed to be dry N dominated if > 50% of the total N was as NH_3-N deposition.

Bryophyte tissue and soluble nitrogen concentrations at the extensive UK sites.

49. A weak but significant relationship was found for both tissue N content and soluble NH_4-N concentration with atmospheric NH_3 concentration for the pleurocarpous mosses sampled in the UK extensive study. There was also a relationship between both indicators and total N deposition. The correlations were higher in response to NH_3 concentration than in response to N deposition, but overall the data showed a higher scatter, which may be due to the fact that the sites were all subject to diffuse sources of N deposition, providing a smaller N deposition range for comparison.

50. Comparing the foliar N content and soluble NH_4-N concentration data for the two most frequently sampled mosses in the UK study (*R. squarrosus* and *S. purum*) shows that there are species differences in response to NH_3 concentrations and N deposition. *R. squarrosus* shows a similar relationship between both NH_3 concentration and N deposition, whereas *S. purum* shows weaker relationship for N deposition and no relationship at all with NH_3 concentration.

51. By contrast, there was a reasonable relationship between total N content and soluble NH_4-N concentration using all the UK site data (R^2= 0.484). This would indicate that the measurements reflect real differences in N availability to the bryophytes, and that the weak overall responses in relation to mapped N deposition are due to the other inter-site differences noted above. This demonstrates the benefit of measuring more than one chemical bioindicator simultaneously.

52. More detailed examination of the tissue N content and NH_4-N concentration values at individual sites revealed a number of unexpected values. For example, at Inverpolly/Knockan (one of the cleanest UK sites) values for *Thuidium tamariscinum* were much higher than expected. This indicates that caution may be needed in such an extensive approach, which utilizes a simple collection of sample at one time. This highlights the need for more detailed checking of such values by more intensive sampling at particular sites.

53. The high scatter in the overall relationship between the foliar parameters and N supply indicates that for sites with diffuse sources of N deposition over a modest range, the foliar N concentration and soluble NH_4-N concentration of bryophytes may not on their own provide a reliable predictor for spatial comparisons at one time (according to the level of replication used in this study). By contrast, such methods may be better suited to implementing within more intensive monitoring, such as a) near a local gradient or in b) more detailed long term monitoring, including repeated sampling` over a period of time. Although, the intensive studies in the present project have shown the potential near known N sources, long-term biomonitoring using these methods still needs to be tested.

54. While recognizing the significant scatter in the data from the UK sites, it is possible to compare the chemical bioindicator data to establish critical loads for the habitats. Based on previous intensive studies of the relationship between total N deposition and total foliar N content, a threshold value of 1.3% N was used as an indicator of N impacts at sites. Application of this value to the present sites showed that 20 out of the 32 sites were estimated to be affected by N deposition. This could have long-term problems for integrity of these sites.

55. By comparison, comparing habitat specific critical loads with estimated N deposition for each of the UK sites showed that 23 out of the 32 sites had N deposition above the current load for their specific habitat. While the individual sites identified were not always the same, this overall number is broadly consistent with that estimated by the bioindicator approach. The advantage of the threshold bioindicator value over the comparison of critical loads with estimated N deposition is that it assigns an actual value based on site measurement.

56. This approach may be applied for other bioindicator parameters, such as foliar ammonium. Previously, a threshold value of 20 μg NH_4-N g^{-1} FW was identified as a threshold value for pleurocarpous mosses of woodland (Sutton *et al*. 2004a), however, the extraction methodology used for the present study gives some what smaller values, so a lower value would be more appropriate with the revised sampling protocol (e.g. 6 μg NH_4-N g^{-1} FW).

UK extensive lichen diversity survey

57. The UK extensive survey was restricted to macrophytes on trunks and twigs of a range of available tree species in sites where ammonia is monitored across the UK. Macrophytes were classified as acidophytes and nitrophytes and others. Indices for acidophyte and nitrophyte frequency were estimated for all sites. In addition the Ellenberg nitrophyte scores were used for all species where available.

58. The results showed that there was a strong correlation of lower acidophyte values and increasing nitrophyte values on trunks and twigs with increasing NH_3, and that loss of acidophytes is occurring at lower concentrations of NH_3 than an increase in nitrophytes. The combined index of AV-NV is strongly correlated with increasing NH_3. In areas of high NH_3 deposition nitrophyte values were higher on twigs than on trunks and associated with higher bark pH. The results suggest that a comparison of macrolichens on twigs and trunks allows an evaluation of changes occurring with time.

59. The results of the UK scale lichen assessment support the previous result of Sutton *et al.* (2004a) that the critical level for NH_3 is set too high. In the present UK dataset for twig lichens, nitrophyte (NV) lichen species tend to dominate over acidophyte lichen species (AV) (AV-NV<0) at NH_3 concentrations of above 1-2 μg m^{-3}. Above similar levels of NH_3 exposure trunk values of AV-NV tend are typically reduced to <5. The data point to the need to revise the annual critical level for NH_3 effects on lichens to ~1.5 μg m^{-3}, which is roughly a factor of 5 less than the current value adopted by the UNECE (8 μg m^{-3} annual average).

Interpretation of the nitrogen bioindicator results and relationship to site condition.

60. Using the NH_3 concentration, N deposition and sulphur (S) deposition data and the results from the lichen indicator value survey and moss sampling, a generalised impact assessment of the UK sites was carried out. Using the critical load for each of the selected habitats and the estimated N deposition (kg N ha^{-1} y^{-1}) critical load exceedance was determined for each site.

61. The 32 UK sites were grouped, where possible, into four general habitat types (upland, mixed broadleaved woodland, Atlantic oak woodland and lowland wetland). Using the grouped data for total N content and soluble NH_4-N concentration, a mean N content was determined for each habitat. This mean value was used to estimate whether the individual sites were potentially being impacted by N deposition.

Implications for impacts of N from the sampling at the UK sites.

62. The application of simplified biomonitoring on a UK scale using local field officers to carry out lichen surveys, collect moss, twig and bark samples worked well. Using the field officers allowed a greater number and range of habitats to be sampled. The standard of moss identification and the quality of the sampled material collected was high.

63. It needs to be emphasized that very clear guidance is necessary to ensure agency staff make the best sampling decisions in the field. Sampling decisions, which may appear obvious to an expert, are often difficult for non-experts. A key need is to make clear which are the priority species for sampling (due to better established relationships) and to underline the need for calibration sampling to be undertaken immediately (<20-50 m) adjacent to air monitoring locations due local variability in atmospheric NH_3 levels.

64. It was found that annual rainfall appears to influence the N content of pleurocarpous mosses with increased precipitation reducing the foliar N content in the UK extensive study mosses. This provides a complicating factor to the interpretation of tissue N content response to N deposition. Further work is required to determine the influence of precipitation volume, episodicity of rain events, and precipitation frequency on foliar N concentrations on a UK scale.

65. Using the mean foliar N content and soluble NH_4-N concentrations derived for four habitat types it is possible to determine a mean concentration for the different habitat types using the moss data collected as part of the UK extensive study.

66. The upland moorland and Atlantic oak woodland had the lowest foliar total N content, followed by the lowland wetlands at 1.30% N and finally the mature woodland at 1.45% N. A similar pattern was found for the habitats soluble NH_4-N concentrations, but there was virtually no difference between the lowland wetland and the mature woodland habitats at 7.4 and 7.7µg g^{-1} FW respectively.

67. Twenty-three out of the 32 sites exceeded the critical load for their habitat type, and a similar fraction was identified on the basis of exceeding a critical threshold of total N content of bryophytes of 1.3% N.

Implications for impacts of nitrogen deposition on condition and integrity for four detailed case studies.

68. The results from a) the simplified biomonitoring methods from both the intensive and the UK extensive studies and b) the N and S deposition data were used to assess the likely impacts of N & S deposition on the condition and integrity of four contrasting SSSI sites. If N biomonitors are to be incorporated as part of site assessment and/or monitoring they must be shown to give added value compared to modelling and critical load assessment.

69. The sites selected were 1) a lowland wood with an agricultural NH_3 source (Piddles Wood SSSI), 2) a lowland raised bog (Caldanagh bog ASSI), 3) an Atlantic oak woodland (Ariundle SSSI) and a mixed broadleaved and yew woodland with neutral and calcareous grasslands (Llanymynech and Llynclys Hills SSSI). At each site, the specific attributes defined under CSM for the site interest feature were assessed in relation to the applicability of biomonitoring methods and the atmospheric pollutant inputs. In general, it was difficult to relate directly the biomonitoring method to the site interest feature.

The interest features were not normally specific N or S sensitive plant/lichen species making direct application difficult, especially for those sites with diffuse N sources.

Developing robustness in biomonitoring and the biomonitoring chain.

70. It is possible to envisage different methods as a "biomonitoring chain" from source to ultimate impact of conservation concern: emission, air concentration, deposition, accumulation, biochemical response, visible injury, growth response, species composition change (most sensitive species), species change (designated species). Bearing in mind the interest to obtain information that links source attribution and ultimate effect, the most robust biomonitoring program can therefore be envisaged as one which uses several methods well distributed along the biomonitoring chain.

71. At its simplest, robustness of biomonitoring may be enhanced by the use of several different methods. However, the difficulty of linking nitrogen biomonitoring directly to interest features demonstrates the need for a cross cutting approach to biomonitoring that links changes in biological features with the source of the pollution. Such a linkage can be conceptualized in the "biomonitoring chain" (Figure 1), which notes that monitoring tools available are distributed across the pathway from source to final effect.

72. The different positions of monitoring along the biomonitoring chain (Figure 1) may be envisaged as: 1) emissions, 2) air concentrations, 3) deposition, 4) biochemical accumulation, 5) biochemical response, 6) visible injury 7) growth responses, 8) species composition change of main species within the habitat, 9) species composition change of designated species. It should be noted that not all stages of the chain may occur or be relevant in all contexts. However, a robust program of monitoring, that is able to link species effects to pollution, would include measurements that are well distributed across this chain.

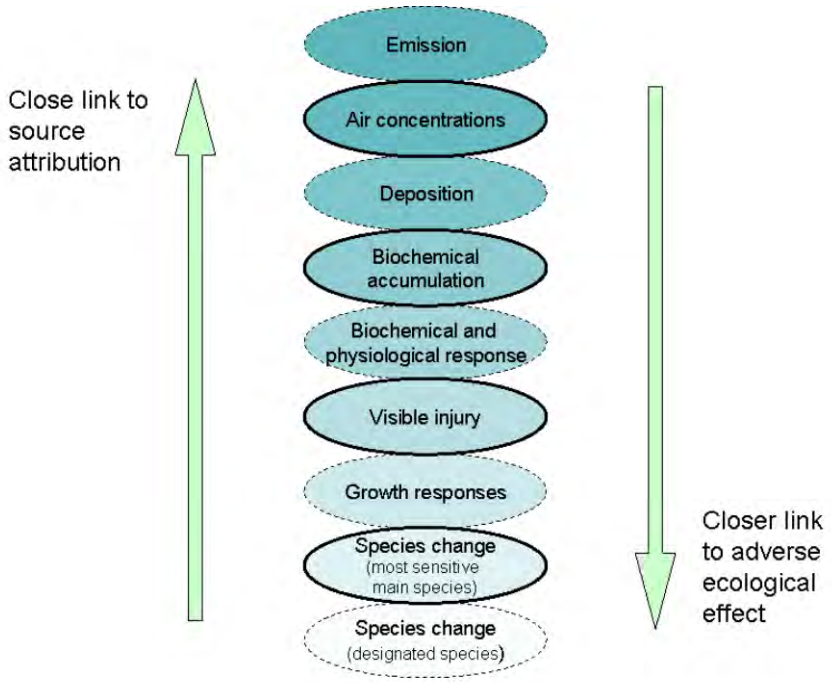

Figure 1. The "biomonitoring chain" demonstrating how use of complementary monitoring methods can help establish the link between source attribution and adverse ecological effect on designated species. Approaches shown with a bold border are in general easier to measure, while those with a dashed border are harder to measure. Not all links in the chain apply in all circumstances.

73. It should be noted that some measurements are easier than others and that these are fortuitously, well distributed along the biomonitoring chain. In particular, a practical program of easier indicator measurements may focus on: 2) air concentrations, 4) biochemical accumulation, 6) visible injury and 8) species composition change of main species within the habitat (the even numbered stages). By contrast, measurement of the odd numbered stages in the chain requires much more resources.

74. The robustness of the biomonitoring chain depends on the logical and observed connections between source and effects. It is obviously most robust to measure all the stages, however, measurement of the even numbered stages should in most cases be sufficient to examine the link between cause and effect.

75. The biomonitoring chain concept provides a helpful tool to guide the practical application of biomonitoring for statutory conservation and environment agencies, as well as helping to identify the challenges. Foremost among the challenges is the extent to which stage 8) and 9) may be linked if it is not feasible to monitor 9) directly. It can be argued that if some species respond to N, then unmeasured designated species may also be at risk.

76. The extent of risk will depend on improving our understanding of the relative sensitivity of the main different species groups and for particular designated species. For example, the success of acidophyte lichens may be relatively independent of the success of different woodland ground flora communities at the same location, and may differ in sensitivity to nitrogen. In such examples, use of different biomonitoring approaches to indicate impacts on the habitat interest features relies on improving the calibration of responses between the indicator and the interest features and N exposure (as air

concentration or N deposition). For this purpose, further refinement is required in "benchmarking" of critical values of the indicators appropriate for different habitats.

77. An example may serve to demonstrate the application of these principles. At Fressingfield in Suffolk, the NH_3 air concentration was measured as 5.3 μg m^{-3}, and the measures of N accumulation in *Eurynchium praelongum*: tissue N content and soluble ammonium concentration were 2.87 total N and 60.4 μg NH_4-N μg g^{-1} FW, respectively. Visible injury for N effects was not assessed. On oak trunks acidophyte (AV) lichens scored 0.7 while nitrophyte (NV) lichen species scored 20.7. On oak twigs, the AV score was 0 and the NV score was 10.4. Overall this provided scores of AV-NV of –20 for trunks and -.10.4 for twigs. Hence both the trunk and lichens indicate a nitrophyte-dominated site. The high values of tissue N content and soluble NH_4-N of bryophytes are somewhat above critical thresholds, indicating a significant amount of N accumulation at this site (although not with the highest values). This is supported by the clear dominance of nitrophyte lichens at this site as compared with acidophyte lichens. The biological measurements therefore point to this site being under threat from atmospheric N, in particular NH_3 (as indicated by the lichen scores). These values are consistent with the N deposition to the site of 41 kg N ha^{-1} y^{-1}, (based on the measured NH_3 concentration and mapped values for other terms), which is larger than the critical load for this habitat (critical load is 20-30 kg N ha^{-1} y^{-1}). Although the selected sampling conducted did not directly demonstrate a loss of favourable condition, the results point to a site under significant threat of N deposition to the integrity of the site with the main source being NH_3 emissions.

78. The example above demonstrates how selected measurements may be used to provide a screening assessment of a site. Where potential problems are identified, there is therefore a need for more intensive measurements, for example using a wider range of approaches along the biomonitoring chain, including more detailed monitoring of the designated species and habitat elements most sensitive to elevated N.

79. Finally, it should be noted that the present short study has necessarily focused on bioindicator methods applied for single sampling periods. Each of the methods increase their robustness when applied repeatedly over time in a planned program of biomonitoring. The two main timescales of biomonitoring for nitrogen may be envisaged as a) short term monitoring (months–a few years) following a local change in conditions (e.g. the impact of emissions from a recent development) and b) long term monitoring following the impact of regional air pollution policies (e.g. several years to decades).

Appendices

Refinement of the foliar ammonium concentration bioindicator method.

80. The development of a simplified extraction procedure for the chemical biomonitoring method, soluble foliar ammonium (NH_4-N) concentration was an integral part of the study. A range of extraction methods and times were tested using: de-ionised water, liquid nitrogen, autoclaving (in water and in sulphuric acid) and ultra-sonic bath.

81. After testing of different extraction solutions and methods using moss tissue, it was established that an extraction time in water of 4 hours, produced levels of NH_4-N at measurable concentrations. This was before significant alternation of the sample with additional ammonium as a product of biological activity took place.

82. The four-hour extraction in water was found to be the quickest, simplest and most cost effective method and produced results, which were comparable with other methods. This extraction procedure was therefore adopted for all soluble NH_4-N concentration measurements of standardised grass and pleurocarpous moss samples from both the intensive and extensive UK studies.

83. The study compared soluble ammonium and nitrate concentrations in moss tissue and established that soluble ammonium concentrations in moss tissue was a better chemical bioindicator than nitrate, which had extremely low measurable concentrations close to the limit of detection.

Application of *Lolium multiflorum* as a standard biomonitor at an intensive site, Whim Moss.

84. The potential application of below ground (roots) biomass and foliar N content as a bioindicator of dry N deposition (along a NH_3 concentration gradient over mire vegetation) was examined using standardised grass biomonitor plants of *Lolium multiflorum*. The above and below-ground biomass and N contents were compared after 2 months of NH_3 exposure (NH_3 concentration range 2-200 µg m^{-3}) at the Whim Moss manipulation facility.

85. *Lolium multiflorum* was found to be a suitable species for use as a standardised grass bioindicator with a defined NH_3 point source under experimental field conditions. The *Lolium multiflorum* above ground and below ground biomass increased significantly with increasing NH_3 concentrations.

86. Although there was an increase in tissue N content in both the above and below ground biomass, the N uptake was greater in the above ground biomass (76%) compared to the root biomass (24%). For the total plant inventory of N, this was dominated by the above ground biomass, accounting for 93% of the overall response.

87. Inorganic clay granules (Agsorb) were tested as a growing medium for *Lolium multiflorum* as compared with normal peat/soil substrate. Use of Agsorb speeded up the extraction/cleaning time of the roots compared to soil/peat based composts although such root extraction is still very time consuming. Each root system took on average 30 minutes to clean.

88. Given the extraction time involved in the determination of root biomass and N content, and the fact that the plant response to N was dominated by the above ground plant material, the use of roots as an N bioindicator is considered not suitable for large scale studies. This simplifies the approach, as in practice it is only necessary to measure the above ground plant parts.

1. Introduction

1.1. Background

Atmospheric nitrogen deposition is a key threat to the integrity of semi-natural ecosystems in the UK that are protected under both UK and European legislation. The principle cause of change is recognized to be through the eutrophication effect of additional nitrogen, which alters the competitive balance between different species. Further additional impacts include the contribution of nitrogen to the acidification of soils and freshwaters, as well as the direct effects of individual components contributing to nitrogen deposition (NEGTAP 2001).

While the effects of deposition on eutrophication and acidification have been assessed in the past using the critical loads approach, the direct effects on vegetation have been assessed using critical levels of concentrations. Critical levels have been established for oxides of nitrogen (NO_x), ammonia ($NH_{3)}$ and cloud water acidity (Achermann and Bobbink 2003). In the UK, reduced nitrogen is now the dominant component of total nitrogen deposition. There is limited field evidence to suggest that given a particular dose of N (as kg N ha^{-1} y^{-1}), the severity of effect on vegetation is NH_3 (gas) > NH_4 (wet) (Leith *et al.* 2001). This may be in contrast with the fact that current critical level (8 µg m^{-3}) for NH_3, which is rarely exceeded at typical concentration in the UK (except close to point sources) and therefore would imply only limited direct effect of NH_3. However, recent evidence suggests that this value is too high (e.g. Pitcairn *et al*. 2003, Sutton *et al*. 2004b). Hence a range of different N containing pollutants appear to be affecting semi-natural ecosystems in the UK, while there remains a key question regarding the relative contribution to these impacts from different N forms.

The Habitats Regulations introduces new site safeguard commitments for the conservation agencies and environmental agencies (i.e. Environment Agency (EA), Scottish Environmental Protection Agency (SEPA), Environment and Heritage Service (EHS)). There are also legislative and policy commitments for the protection of designated sites in the UK. Nitrogen deposition and concentrations are a threat to the condition and long-term maintenance of sensitive habitats and species.

The current policy assessment for atmospheric N deposition impacts at a UK scale utilises the critical loads and levels approach, in particular the use of N deposition maps across the UK in conjunction with critical loads exceedance, associated with mapped distribution of different habitat types. This national mapping approach can be used to give an initial estimate of critical loads and levels exceedance at specific designated sites (e.g. SACs, SSSIs). The approach is currently used by the conservation and environmental agencies as a risk assessment of air pollution impacts on the integrity of designated sites. The critical loads approach is, however, limited to indicating an increased risk of environmental impact as exceedance of critical loads does not equate exactly to an impact on ‘site condition’. Furthermore, there are limitations with the resolution of the deposition modelling and habitat and soils mapping which means there are many uncertainties in applying such an approach at an individual site level.

As part of the statutory role of the conservation agencies the condition of designated nature conservation sites is monitored under the Common Standards Monitoring (CSM) programme. The CSM provides an assessment of the condition of interest features on SSSIs as a single snapshot in time, which presently does not include a predictive assessment of the long-term risk to sites. The use of nitrogen biomonitors, either as a single measurement or repeated

measurements over time could complement the condition monitoring assessments and provide an indication of the impact of N deposition on a specific site and the potential impacts on the long term integrity of the site/interest features. Although in most cases any applied biomonitor method may not target specifically the interest features of the designated site, it could provide appropriate information which would indicate the potential for N pollution impacts on the site as a whole.

Bioindication and biomonitoring represent a complementary approach to the assessment of air pollution impacts of nitrogen. Bioindication for atmospheric nitrogen represents the use of biological measurements on a specific site of interest to indicate either a level of **exposure** (N deposition or concentration) and/or **ecosystem impact**. Where bioindicators are well characterized, they should be able to provide quantitative information from measurements conducted at a site at any given time regarding the level of exposure or impact associated with the site condition. Biomonitoring represents the extension of bioindication to consider the status of the site through time, and therefore represents the repeated application of given bioindicator methods.

To identify generalised N biomonitoring techniques which could be used as complementary tools in the condition monitoring of the statutory sites, Sutton *et al*. (2004a) provided a comprehensive review of existing N bioindicator methods. The objective was to identify those methods, which would provide an early indication of N effect, N exposure or ecological impact on statutory nature conservation sites. The applicability of the identified methods for general biomonitoring was assessed by empirically scoring the different methods for general application, according to criteria reflecting i) robustness, ii) ease of use and iii) extent of method development/establishment. In addition, a decision approach reflecting the different specific potential purposes of the bio-monitoring methods was also used.

Seven methods were identified from the empirical selection process as being the most suitable for general application. These were:

Chemical methods

- Total foliar N concentration (primarily of bryophytes, but also of higher plants).
- Soluble foliar N concentration (evaluating foliar ammonium, nitrate and total soluble nitrogen).
- Measurement of the pH of tree trunks and twigs (as a supporting parameter to help interpret lichen diversity measurement; see below).

Diversity methods

- Ellenberg nitrogen index values of higher plants and bryophytes
- Measures of lichen diversity in relation to nitrogen preferences (using the van Herk and Wirth approaches), applied for twigs and trunks of trees.

Transplant methods

- Standardised model plants (e.g. *Lolium* spp., *Deschampsia* spp.) grown under standard conditions, and then exposed at monitoring, followed by measurement of growth and N inventory.
- Native reciprocal transplants (e.g. bryophytes), where natural plant material is exchanged between sites of varying N exposure, followed by measurement of growth and N inventory.

It was noted that each of these methods would benefit from further testing and application in the UK, and these form the basis of methods tested in the present study. It was recognized that the last of these (native reciprocal transplants, e.g. Mitchell *et al.* 2004) is more resource demanding than the others, and this approach was therefore not tested in the present project.

1.2. Objectives of the project

The main objective of the project was to refine, test and subsequently recommend bioindicator/biomonitoring methods for assessing the impacts of atmospheric nitrogen deposition or concentrations on statutory nature conservation sites. To do this, the work focused on contrasting situations with different habitat types and atmospheric pollutant combinations, and also included development work to refine the procedures for measurement of soluble foliar nitrogen, as a bioindicator of N pollution.

This study should help the conservation and environmental agencies to better understand the effects of nitrogen on site condition and integrity. These biomonitoring methods should also help validate the use of critical loads in providing a national overview of risk to the site series.

1.3. Report Field studies

The two main field components of the project were:

1) To test selected bioindicator methods in detail at four key contrasting sites.
The four sites referred to as the "intensive" sites were:

Piddles Wood, Dorset, a lowland woodland with dry deposition of agricultural NH_3 as the main source of nitrogen pollutant:

Happendon Wood, Lanarkshire, a wooded site adjacent to the M74 motorway with dry deposition of vehicular NO_x (plus associated NH_3) as the pollutant of interest.

Bowbeat Hill, a high altitude moorland dominated by wet deposition of N from NH_x and NO_y. This site is contrasted with a nearby low altitude moorland, **Auchencorth Moss**, with lower wet deposition inputs.

Whim Moss a nitrogen manipulation study on a *Calluna vulgaris*/*Eriophorum vaginatum* mire comparing the effect of different forms of N deposition (dry NH_3, vs. wet NO_3^- vs. NH_4^+).

2) To apply the simplified methods validated in (1) at the UK scale using sites with existing robust measurements of atmospheric N exposure.

Overall thirty-two sites (referred to as the "extensive" sites) were selected covering the devolved regions of the UK.

Two other objectives were:

3) To refine the soluble nitrogen methodology and apply it at the intensive and UK extensive sites.

4) To synthesise, using the results from the Intensive and UK Extensive sites, the applicability of N biomonitors and biomonitoring for the conservation agencies, SEPA and EHS in their condition monitoring and integrity assessment of designated sites (i.e. SSSIs, SACs etc).

1.4. Structure of the report

This report describes the results of the different bioindicator methods tested at both the intensive and UK extensive sites and gives an overview of their general applicability to conservation agencies, SEPA and EHS.

The study was divided into four main components a) development of the methodology for soluble NH_4-N concentration determination, focusing on the measurement of foliar ammonium concentrations, (Appendix I) b) the testing of simplified biomonitoring methods in parallel at four intensive sites (Sections 3-8) c) the application of selected simplified methods at the UK scale in the extensive study (Sections 9-13) and d) applying the results from the intensive and extensive to assess N impacts on condition and integrity of SSSI's and production of protocols and 'Bench marking' for specified habitat/pollutant scenarios (Sections 14, 15 and 16).

2. Measurement methods for monitoring atmospheric nitrogen compounds at the study sites

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2.1. Introduction

An important criteria for selecting the extensive UK-sites was the availability of atmospheric measurements, which would provide nitrogen deposition estimates for the study sites. This is important since at a site level there is substantial sub-grid variability in atmospheric nitrogen deposition, and therefore applying national mapped estimates at a site level introduces a high degree of uncertainty (Sutton *et al*. 2003). In seeking to reduce these uncertainties in the most cost-effective way, it may be noted that the spatial uncertainties differ for different N components. Therefore, uncertainties may be reduced by a combination of measured parameters combined with some mapped estimates. Firstly the most spatially uncertain component, which also contributes the largest to overall nitrogen deposition, is atmospheric ammonia (NH_3). Secondly, in the vicinity of major sources, such as roads, nitrogen dioxide can contribute substantially to the uncertainty in estimated nitrogen deposition. Thirdly, in hill and mountain areas there is a high spatial variability in wet deposition of nitrogen, making it important to have reliable measurements of precipitation chemistry and overall precipitation amounts for sites in such areas. By contrast, the available evidence suggests that concentrations of aerosols and other secondary components such as nitric acid are less spatially variable, and estimates can be made from national datasets.

The requirement for air concentration and deposition measurements at a site level is dealt with in this study through a combination of existing and supplementary measurements where these were missing, combined with mapped estimates of the slowly varying terms. At the intensive study sites additional measurements of ammonia concentrations were made, supplemented by measurements of nitrogen dioxide for the site adjacent to a major road (Happendon Wood). For the sites assessing the effects of high and low wet deposition, the measurement effort required that the sites be selected where existing measurements are already available (Bowbeat Hill, Auchencorth Moss). Finally, for the extensive survey of UK sites, these were as far as possible selected at sites where ammonia and other N deposition components were already or previously measured (see Section 11).

In this section the air measurement methods as applied in this study for ammonia and nitrogen dioxide are described.

2.2. Measurement methods

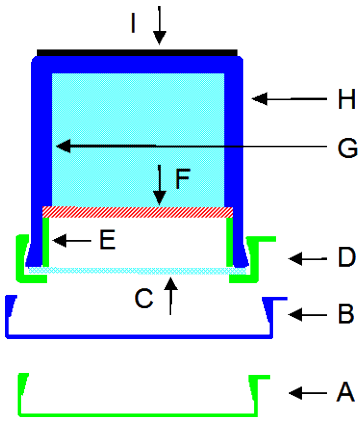
2.2.1. Ammonia concentration measurements at extensive UK sites

The ammonia concentration for the UK sites in the extensive study (section 11) was known because they are part of or are located very close to sites in the UK National Ammonia Monitoring Network (UK NAMN, started in 1996, with over 90 sites: www.cara.ceh.ac.uk/networks), the nitric acid network (HNO_3-network, started in 1999, with 12 sites: www.cara.ceh.ac.uk/networks), Ammonia monitoring in Northern Ireland (NI, April 2003-March 2004: Tang *et al*. 2004) or in the GANE Epiphyte project (Mitchell *et al.* 2005). Details of the study sites are shown in Table 2.1.

Table 2.1. Extensive UK sites: ammonia measurements that were made in ongoing networks or in other projects.

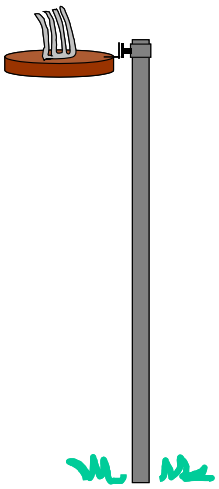
Site no.	Site Name	National Ammonia Monitoring Network	National HNO_3 Monitoring Network	NI Ammonia Monitoring Network (SNIFFER)	GANE Epiphytes Project (Mitchell *et al*. 2004)
1	Cwmystwyth	X	X		
2	Plynlimon	X			
3	Dyffrn Mymbyr	X			
4	Stackpole/Orielton	X			
5	Eskdalemuir	X	X		
6	Halladale	X			
7	Strathvaich Dam	X	X		
8	Glensaugh	X	X		
9	Inverpolly/Knockan	X			
10	Edinburgh Centre	X	X		
11	Ariundle				X
12	Glen Nant				X
13	Wood of Cree				X
14	Bush	X	X		
15	Sherwood (Ladybower)	X			
16	Moorhouse	X			
17	Fenns' Moss B	X			
18	Stanford	X			
19	Fressingfield	X			
20	Bedlington/Bedingfield	X			
21	Borrowdale				X
22	Brown Moss	X			
23	Llynclys Common	X			
24	Wytham Wood	X			
25	Lullington Heath	X			
26	Lough Navar	X	X	X	
27	Glenmore Wood			X	
28	Caldanagh Bog			X	
29	Castle Enigan			X	
30	Orritor			X	
31	Redgrave and Lopham	X			
32	Yarner Wood	X			

For the NAMN at sites where electricity was available the DELTA (DEnuder for Long-Term Atmospheric sampling) for sampling ammonia and ammonium was used. This is a low-cost diffusion denuder system that was developed for long-term sampling of atmospheric ammonia and ammonium (Sutton *et al*. 2001). At sites without electricity the new improved high sensitivity CEH ALPHA (Adapted Low-cost Passive High Absorption) sampler (Tang *et al.* 2001) (see Figures 2.1 and 2.2) was used for atmospheric ammonia concentrations.



A Replacement solid cap
B Top protective cap
C 5 µm PTFE membrane (27 mm diameter)
D Cap with hole for membrane
E Support ring (6 mm height)
F Filter paper
G Internal ridge to support filter paper
H Extended body for ease of handling
I Velcro for attachment to holder

Figure 2 1. Outline diagram of a single ALPHA Sampler used for measuring ammonia concentrations.



The passive sampling system consists of three replicate ALPHA samplers attached by velcro to an aerodynamically shaped support (plant saucer) on a pole or post at about 1.5 m height above ground / vegetation (Figure 2.2). A strip of aluminium sheet, partly cut into small strips, is mounted on top of the support to deter birds from perching. Two metre galvanized metal posts are used which is sunk 0.5 m into the ground and brackets are used for securing the support to the post. Triplicate samplers are used in order to give a more reliable estimation of the air concentration of ammonia.

Figure 2.2. ALPHA sampler support on a metal post.

In the HNO_3 network the same DELTA-system is used. For the Northern Ireland sites, which were not part of the NAMN and the GANE Epiphyte sites the ALPHA sampling system (Figures 2.1 and 2.2) was used.

Ammonium captured on the samplers was analysed on the AMFIA (AMmonia Flow Injection Analyser), which is based on selective dialysis of ammonium across a membrane at high pH with subsequent analysis of conductivity. With these results the ammonia air concentrations were calculated.

2.2.2. Air monitoring at the intensive sites

Ammonia concentrations for Whim Moss and Auchencorth Moss were available from ongoing CEH projects. As air concentrations measurements were not routinely measured at the intensive sites Piddles Wood (NH_3), Happendon Wood (NH_3 and NO_2) and Bowbeat Hill (NH_3), additional air monitoring was carried out at these sites for a 3 month period (Section 3). The locations, distances from source and the number of the sampling points at the intensive sites are fully described in Section 3.

2.2.3. Ammonia concentration measurements

Ammonia was measured at Piddles Wood, Happendon Wood and Bowbeat Hill using CEH ALPHA samplers. At Piddles Wood, there were five air monitoring points along a NH_3 gradient away from the poultry unit (Figure 2.3).

Figure 2.3. Ammonia monitoring at Piddles Wood 40 m from the poultry unit.

At Happendon Wood, there were six air monitoring points at various distances away from the M74 motorway. At Bowbeat Hill there was one monitoring point. At these 3 sites, air was monitored for a total of 3 months (2 x 6 weeks exposure periods).
The intensive site, Auchencorth Moss is part of the NAMN, so no additional air monitoring was carried out.

At Whim Moss the NH_3 concentrations were monitored using a combination of diffusion tubes and ALPHA samplers at 2 heights above the vegetation (0.1 and 0.5 m) along a 60 m NH_3 concentration gradient from a 10 m line source as part of a study investigating the effects of different forms of N deposition on vegetation. All samplers were changed monthly.

2.2.4. NO_2 concentration measurements

Atmospheric NO_2 concentrations were monitored (Happendon Wood) using a modification of the 7.1 GRADKO passive diffusion tube (Tang *et al*. 2002), with analysis of nitrite by a modified Griess-Saltzman procedure (Hargreaves 1989). The modification involves insertion of a PTFE membrane across the open end of the diffusion tube during sampling, preventing ingress of insects, and reducing the effects of air turbulence. The NO_2 passive sampling system consists of 3 replicate tubes mounted in clips inside a specially designed support. The support shelters the samples from wind and rain, and also from sunlight. The support with these diffusion tubes was attached to the same post as the support for the ammonia ALPHA samplers.

2.2.5. Accuracy

The accuracy of the analytical methods is assured by participation in the Aquacheck ‘International Proficiency Testing and Benchmarking for Analytical Laboratories’. As part of an ongoing validation programme for the passive methods, the passive samplers are also continuously assessed against reference methods.

2.3. Results

The ammonia air concentrations measured during the experiment are shown in Table 3.2 (Piddles Wood), Table 3.4 (Happendon Wood) and Table 3.7 (Bowbeat Hill) in Section 3 (Intensive sites: site descriptions and atmospheric N monitoring data).

3. Intensive sites: site descriptions and atmospheric N monitoring data

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3.1. Introduction

The need to refine and test biomonitoring methods by applying them in parallel at sites with different habitats, atmospheric N pollutants (i.e. forms of pollutants) and deposition inputs was identified by Sutton *et al*. (2004a). The range of habitats, N pollutant combinations and investigative methods selected for investigation in this current study were

a) lowland oak woodland with an agricultural pollutant source (NH_3) using methods 1-7,

b) woodland situated next to a road as a point source pollutant (road side effects) using methods 1-7,

c) upland moorland exposed to enhanced wet deposition and a lower altitude equivalent wet deposition site using methods 1-7 and

d) experimental N manipulation study on a *Calluna vulgaris*/*Eriophorum vaginatum* dominated mire (NVC; M19a) (Table 3.1).

This section details site and botanical description, NH_3 concentration and N inputs for each of the selected intensive sites.

Table 3.1. Descriptions of the habitats, sources and methods applied at each of the intensive sites.

Methods			**1**	**2**	**3**	**4**	**5+6**	**7**
Habitat	**Source of N**	**Location**	**Tissue N**	**Soluble N**	**Bark pH**	**Ellenberg**	**lichen spp.**	**Grass Biomonitors**
Lowland wood / bog	Farm (NH_3)	Piddles Wood Dorset	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**
Road verge/ wood	Road (mainly NO_x)	M74/ Lanarkshire	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**
Lowland site	Wet deposition	Auchencorth Moss	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**
Hill/ forest	Wet deposition	Bowbeat Hill	**Y**	**Y**	**Y**	**Y**	**Y**	**Y**
Moorland	Different N forms compared	Whim Moss	**Y**	**Y**	**Not Available**		**Y**	**Y**

3.2. Piddles Wood

3.2.1. Site Description

Piddles Wood (NGR: ST790128: altitude 100 m above sea-level) is a designated SSSI immediately south of the River Stour and close to the village of Sturminster Newton in north Dorset (Figure 3.1). The woodland (total area of 62.2 hectares) is divided into four units (Figure 3.2). A poultry unit (100 m long) and housing approximately 100,000 birds is situated adjacent to the edge of the woodland (known as Unit 2) (Figure 3.2). The poultry unit has been operational for approximately 10 years (Figure 3.3). The woodland site is a popular local amenity with a network of footpaths, which are regularly used by local walkers. The site was regularly visited throughout the period (26 April-21 July 2004) by a local environmental consultant on our behalf to check on the standardised grass plants and to change the NH_3 ALPHA samplers.

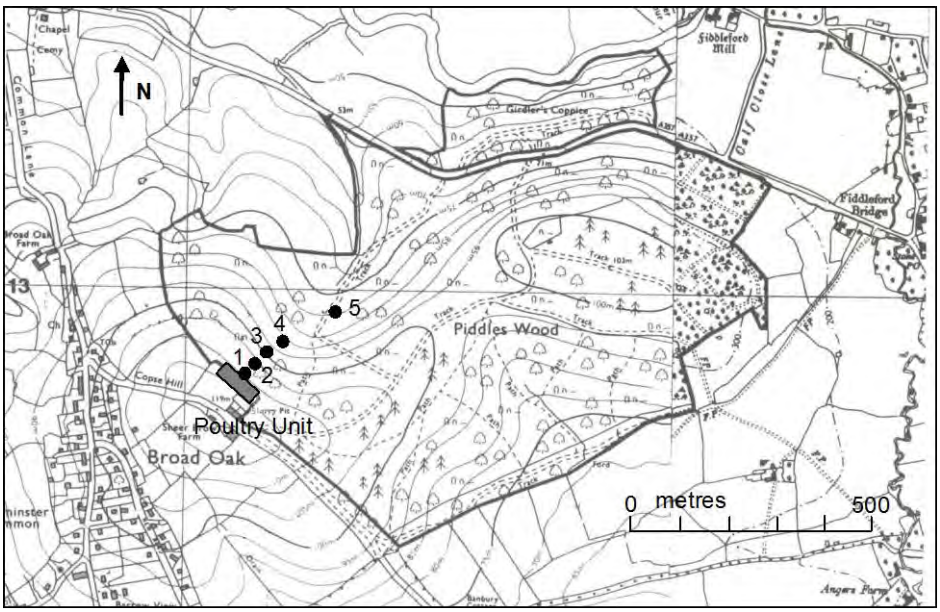

Figure 3.1. Map of Piddles Wood, north Dorset showing the location of the NH_3 transect in relation to the ~ 100,000 bird poultry unit. An additional site (location 5) was established on 8 June 2004 with an exposure period (8/06/04-21/07/04).

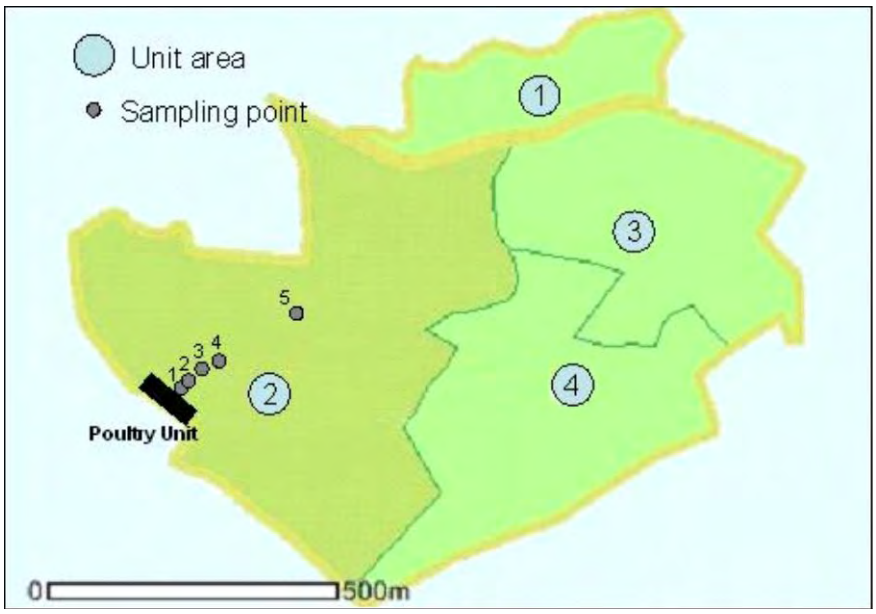

Figure 3.2. Map of Piddles Wood showing the 4 woodland units and the location of the monitoring and sampling sites (Adapted from www.english-nature.org.uk/special/sssi/).

3.2.2. Botanical Description

Piddles Wood SSSI

Piddles Wood is a lowland woodland dominated by a mix of broadleaved species and yew covering an area of 62.2 ha. The woodland was designated SSSI status in 1985 because of its substantial oak woodland and coppiced hazel understorey. The traditional method of growing oak as standard trees with coppice beneath, although no longer economic, has been continued as a method of preserving an open, species rich woodland. A 'coppice with standards' oak wood provides a much lighter shade than a mature closed canopy, hence enabling a shrub understorey to develop. The understorey has been coppiced at regular intervals, thus renewing the vigour of the ground flora.

The woodland thus supports a range of plant communities and associated fauna typical of oak woodland in Dorset. On the lower lying Kimmeridge Clay area closer to the river, the tree layer is composed of moderate aged, tall, vigorous oak with locally occurring ash (*Fraxinus excelsior* and aspen (*Populus tremula)* while the understorey is dominated by hazel (*Corylus avellana*) with some Maple (*Acer campestre*) and Wild service tree (*Sorbus torminalis*) and supports a very rich ground flora. The higher areas on Plateau Gravel also support Oak woodland with Ash and maple but with sparser hazel understorey and a very different ground flora (extracted from www.englishnature.org.uk/special/sssi/sitedocuments.cfm.)

The study area (highlighted in Figure 3.2 as Unit 2 of the woodland area) lies largely on Plateau Gravel with lighter generally acid soils although areas of calcareous soil also occur. At the top of the slope on a well drained area closer to the poultry unit (Figure 3.3) the bluebell (*Hyacinthoides non-scripta*) is locally dominant with ivy (*Hedera helix*); ground ivy (*Glechoma hederacea*) and yellow archangel (*Lamiastrum galeobdolon*) are also locally abundant with locally frequent *Stellaria holostea* and Chervil (*Chaerophyllum sylvatica*). Further down the slope, dog's mercury (*Mercurialis perennis),* Enchanters nightshade (*Circea lutetiana*), *Carex sylvatica* and *Poa trivilais* are locally frequent. *Dactylorhiza fuchsii* and *Conopodium majus* were occasional to rare and many species associated with damp oak wood were also present. At the foot of the slope 100 m from the poultry unit, hazel was cut in 1990, resulting in locally dense areas of bramble (*Rubus fructicosus*) and bracken (*Pteridium*

aquilinum). In 2004, in more open areas, bracken was dominant and whilst bramble and nettle (*Urtica dioica*) were common. Ferns and bryophytes were abundant throughout the transect including *Eurynchium praelongum, E. striatum, Atrichum undulatum, Thuidium tamariscinum, Thamnium alopecurum, Fissidens bryoides, Mnium undulatum, Mnium hornum* and *Plagiothecium denticulatum.*

This type of woodland community termed *Quercetum roburis* by Tansley (1911) falls into the NVC class W8 – *Fraxinus excelsior - Acer campestre - Mercurialis perennis* woodland (Rodwell 1991). The community is very diverse and seven sub-communities have been described. The *Hedera helix* sub-community occurs in some parts of the study area, as would be expected in south-west Britain while in other areas the floristic composition is more typical of the *Primula vulgaris – Glechoma hederacea* sub-community.

For condition assessments, the woodland has been divided into 4 units (Figure 3.2). In July 1998, Unit 2, incorporating the study area was assessed as "Unfavourable recovering". Regrowth of coppice in clear fellings on the plateau gravels (2 x 0.5 ha) had failed due to deer grazing. Regrowth was satisfactory however on lower Kimmeridge clay slopes. In general, the age of mature trees is very uniform and was considered as being in need of diversifying the age structure.

Figure 3.3. Photograph of the monitoring /sampling site number 1 showing the close proximity of the poultry unit to the edge of the woodland.

Figure 3.4 shows site 4 photographed in April 2004 and again in June 2004. It illustrates the change in vegetation cover and shading between spring and summer. This change in cover density was important in the *Deschampsia flexuosa* study and it also highlights the importance of including seasonality in any biomonitoring studies.

Figure 3.4. Photographs of Piddles Wood at 100 m from the poultry unit taken on 26 April and 21 July 2004 showing the seasonal variation in vegetation cover and especially Bracken (*Pteridum aquilinum*).

3.2.3. Summary of NH_3 concentration

The ammonia air concentrations measured during the experiment are shown in Table 3.2. The concentrations are very high close to the poultry unit decreasing with distance away from the poultry unit.

Table 3.2. Ammonia air concentrations (µg m^{-3}) at Piddles Wood from 27-04-2004 until 21-07-2004 at different distances from the poultry unit.

	Distance (m) from poultry unit	NH_3 (µg m^{-3})		
		27/04 – 08/06	08/06 –21/07	Average
Site 1	5	123	79.4	101
Site 2	20	27.9	34.7	31.4
Site 3	40	14.7	19.5	17.1
Site 4	100	1.94	2.0	2.0
Site 5	250		1.5	1.5*

* Only exposed from 23/06/04–21/07/04

3.2.4. Summary of N deposition

The total N deposition has been estimated for each of the five distances away from the poultry unit (Table 3.3). These N deposition estimates were derived from the NH_3 concentration measurement data and the map estimated deposition of nitric acid, NO_2 and wet N deposition (NEGTAP 2001; Smith 2000).

Table 3.3. Estimated total annual N deposition for along a 250 m transect away from a poultry unit at Piddles wood, north Dorset.

Distance from poultry unit	Total estimated N deposition
Metres	kg N ha^{-1} y^{-1}
5	1062
20	337
40	189
100	31.3
250	26.2

The very high values could result in cuticular saturation, which might mean that the full deposition rate near the source may not be achieved.

3.3. Happendon Wood

3.3.1. Site description

Happendon Wood site is a woodland complex on the edge of the M74 motorway in south Lanarkshire, Scotland (NGR: NS 855335, altitude 214 m above sea-level). The M74 is the main Scottish motorway link with the northwest of England. It has an average flow of vehicles of 35,000 vehicles per day and a maximum flow of 85,000 (DfT 2003). The study site located to the northeast of the M74 motorway is intersected by the B7078 into two distinct vegetation areas (Figure 3.5). The woodland is within 10 m of the hard shoulder of the motorway. There is a small wastewater pumping plant (Figure 3.5: position 3), but it did not appear to be continuously operational. A 250 m transect was set up running from the edge of the motorway. The transect was initially set-up with five sites (locations 1-5: Figure 3.5) for NH_3/NO_2 monitoring and standardised *D. flexuosa* plants. An additional monitoring site (location 6) was established mid-way through the 12-week period, on the recommendation of Dr P Wolseley (NHM) following her lichen survey of the site. The purpose of the additional site was to determine the influence of the pollutant inputs from the B7078 on the lichen diversity and moss species composition and N status.

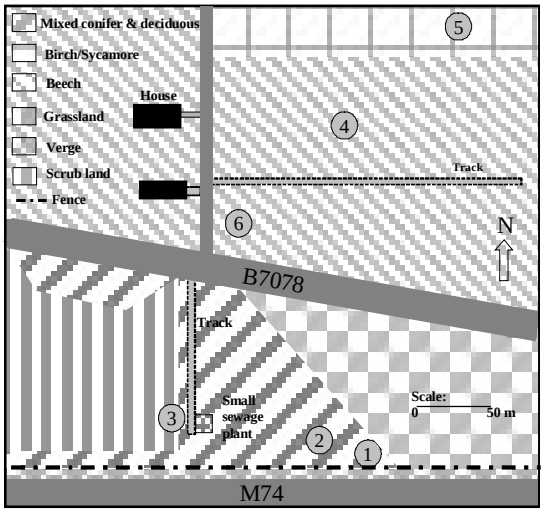

Figure 3.5. Schematic diagram of Happendon Wood showing locations of the sampling sites and the vegetation types.

3.3.2. Botanical description

Happendon Wood in South Lanarkshire is a split site. One part comprises mixed conifer woodland along the M74 motorway (Figure 3.6a) and the other part comprises mature beech/oak Woodland some 200 m from the M74 (Figure 3.6b). The woodland is not continuous, and between the 2 parts runs the B7078 a dual carriageway. Close to the M74, the woodland canopy is fairly dense with light grass cover (*Holcus mollis, Festuca rubra*) and abundant bryophytes. In more open areas, raspberry (*Rubus idaeus*), rose-bay willow herb (*Chamaenerion angustifolium*) nettle (*Urtica dioica*) and *Holcus mollis* are locally abundant and bryophyte cover is dominated by *Scleropodium purum* and *Rhytidiadelphus squarrosus.*

The mature beech woodland supports a limited ground flora due to fairly heavy leaf litter. Like most shelterbelts of beech, the area is exposed to wind on one edge and the ground flora is dominated by grasses. *Holcus mollis* and *Deschampsia flexuosa* are locally abundant, with

the former more frequent in the less exposed areas and common bryophytes include *Rhytidiadelphus loreus, R. triquetrus, Dicranum scoparium* and *Polytrichum formosum*. Where the woodland borders on the B7078, *Galium aparine, Veronica chamaedrys* and *Viola riviniana* are locally frequent and *Anemone nemorosa, Conopodium majus* and *Succisa pratensis* are occasional. The area most distant from the M74 has a mixed canopy of *Larix decidua, Betula pendula* and *Pinus sylvestris* and the ground is damper and more basic. *Holcus mollis* and *Juncus effusus* are locally abundant; *Agrostis stolonifera, Anthoxanthum odoratum,* and *Molinia caerulea* are frequent with occasionals including *Lotus uliginosus, Lathyrus pratensis* and *Lynchis flos-cuculi.* The planted beech wood fits in approximately with NVC class W15, *Fagus sylvatica–Deshampsia flexuosa.*

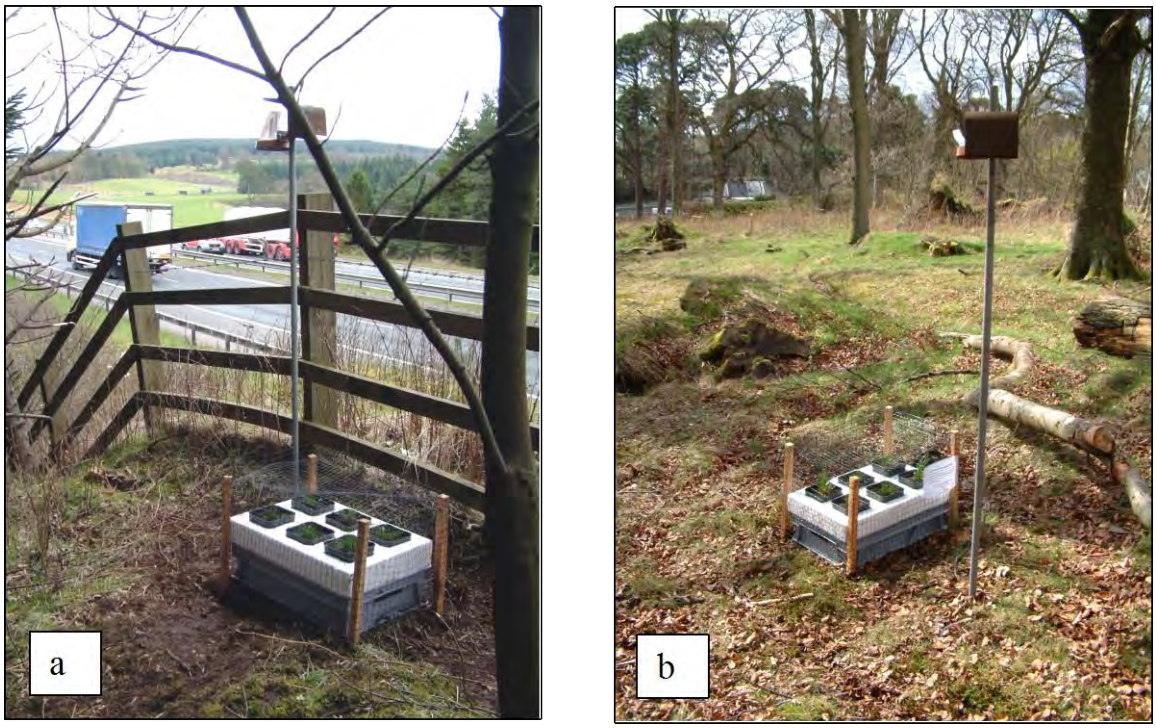

Figure 3.6. Happendon Wood: a) Site 1 close to the edge of the M74 motorway and b) Site 4 with beech, oak and sycamore woodland looking southwest towards M74 motorway.

3.3.3. Summary of NH_3 and NO_2 concentration

The ammonia air concentrations measured during the experiment are shown in Table 3.4. The concentrations at site 3 are slightly higher than at site 2 although, it is further from the motorway. This may be due to the influence of the small wastewater pumping station located near site 3.

Table 3.4. Ammonia and NO_2 concentrations (µg m^{-3}) at Happendon Wood from 20-04-2004 until 12-07-2004 at different distances from the M74 motorway.

Site and distance from B7078	Distance (m) from motorway	NH_3 (µg m^{-3})			NO_2 (µg m^{-3})		
		20/4- 03/06	03/06-12/07	Average	20/4- 03/06	03/06-12/07	Average
1 150 m	10	1.9	0.6	1.3	18.7	8.1	13.4
2 140 m	20	0.5	0.5	0.5	15.7	7.4	11.5
3 120 m	38	1.1	0.8	0.9	19.3	8.6	13.9
4 80 m	200	0.5	0.5	0.5	8.9	3.8	6.3
5 130 m	250	0.4	0.3	0.4	8.1	3.0	5.6
6 20 m	150	n.d.	0.5	0.5*	n.d.	4.8	8.5*

* Estimated for whole period based on comparison for 3 June to 12 July 2004.

3.3.4. Summary of N deposition

The total N deposition has been estimated for each of the six distances away from the M74 motorway. These N deposition estimates were derived from the NH_3 and NO_2 concentration measurement data and the estimated UK mapped deposition of nitric acid and wet N deposition (Table 3.5).

Table 3.5. Estimated total annual N deposition for a 250 m transect away from the M74 motorway at Happendon Wood, south Lanarkshire.

Site	Distance from M74 motorway	NH_3-N deposition	NO_2-N deposition	Wet dep. & HNO_3	Total estimated N deposition
	metres	kg N ha^{-1} y^{-1}	kg N ha^{-1} y^{-1}	kg N ha^{-1} y^{-1}	kg N ha^{-1} y^{-1}
1	10	13.2	2.6	6	22.8
2	20	5.1	2.2	6	14.3
3	38	9.7	2.7	6	19.4
6	150	5.0	1.2	6	13.2
4	200	3.6	1.1	6	11.7
5	250	4.7	0.9	6	12.6

3.4. Auchencorth Moss

3.4.1. Site Description

Auchencorth Moss (NGR:NT220562; altitude 260 m above sea-level), which was selected as the lower altitude moorland site for comparison of wet deposition inputs, is situated 12 km from CEH Edinburgh in the Scottish Borders (Figure 3.7). Auchencorth Moss is part of a larger moorland area part of which is used for peat extraction. The field facility at Auchencorth Moss is extensively used by the atmospheric sciences section at CEH Edinburgh to monitor a range of atmospheric pollutants and meteorological parameters (Figure 3.8). The site is also part of the UK National Ammonia Monitoring Network. The relatively flat topography of the site makes it ideal for micro-meteorological gas flux measurements.

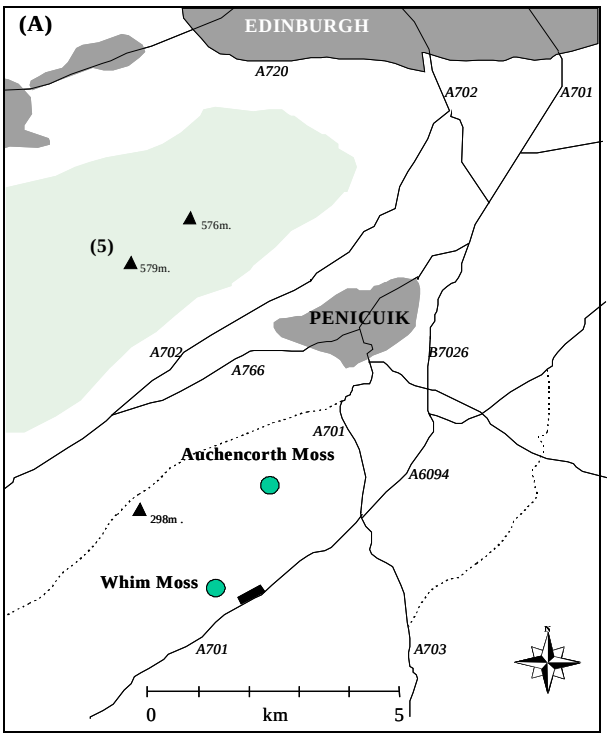

Figure 3.7. Map showing the location of Auchencorth Moss and Whim Moss in SE Scotland**.**

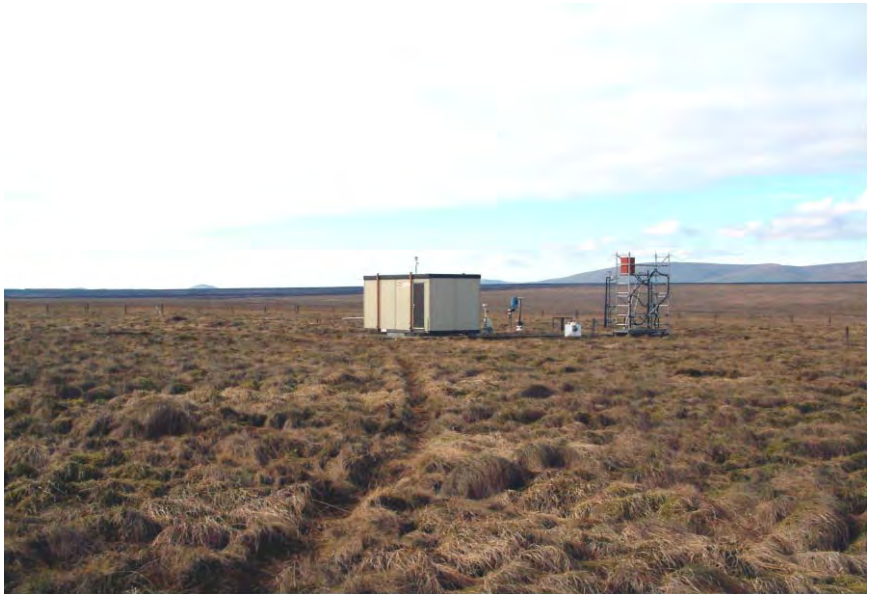

Figure 3.8. Photograph of Auchencorth Moss showing the CEH monitoring cabin and micro meteorological instrumentation.

3.4.2. Botanical description

This lower altitude site (260 m above sea-level) on the eastern borders of a drained valley bog was included to compare the effect of altitude on N wet deposition and vegetation composition. This site is part of a large valley bog, which has been extensively drained for peat extraction in areas. The presence of a CEH monitoring site within a fenced enclosure allows for recording in ungrazed and grazed areas. The site is currently dominated by *Molinea caerulea* and *Eriophorum vaginatum*, with *Deschampsia flexuosa* and *Juncus squarrosus. Calluna vulgaris is* absent, but *Erica tetralix* occurs occasionally. Bryophyte cover includes

locally frequent *Hylocomium spendens, Pleurozium schreberi, Rhytidiadelphus squarrosus* and *Sphagnum capillifolium.*

Within the ungrazed enclosure, *Juncus squarrosus* is absent and *Galium saxatile* is locally frequent while *Pleurozium schreberi* and *Polytrichum commune* dominate bryophyte cover.

3.4.3. Summary of NH_3 concentration

The average ammonia concentration at Auchencorth Moss (September 1996 – December 2003) was 0.81µg m^{-3}. Ammonia air concentrations during the period (May – July 2004), when standardised grass plants (see Section 5) were exposed to the air, are shown in Table 3.6.

Table 3.6. Ammonia concentrations (µg m^{-3}) at Auchencorth from May – July 2004.

	NH_3 (µg m^{-3})		
Auchencorth Moss	May	June	July
	0.9	0.5	0.8

3.4.4. Summary of N deposition

The annual N deposition at Auchencorth Moss is 13.9 kg N ha^{-1} y^{-1} based on NH_3 concentration measurements and including nitric acid and wet deposition measurements.

3.5. Bowbeat Hill

3.5.1. Site description

The designated upland site for assessing high rates of wet deposition is Bowbeat Hill (NGR: NT283473: altitude 584 m above sea-level), which is a CEH Edinburgh field site in the Scottish Borders. The site on the top of Bowbeat Hill is managed moorland, owned by Rosebury Estates, which has recently been developed by Natural Power Systems Ltd as a wind farm with twenty-four Nordex 1.3 MW wind turbines (Figures 3.9 and 3.10). CEH Edinburgh currently monitor/measure precipitation volumes and chemistry, heavy metals and meteorological parameters at this site. The site is visited weekly by CEH staff.

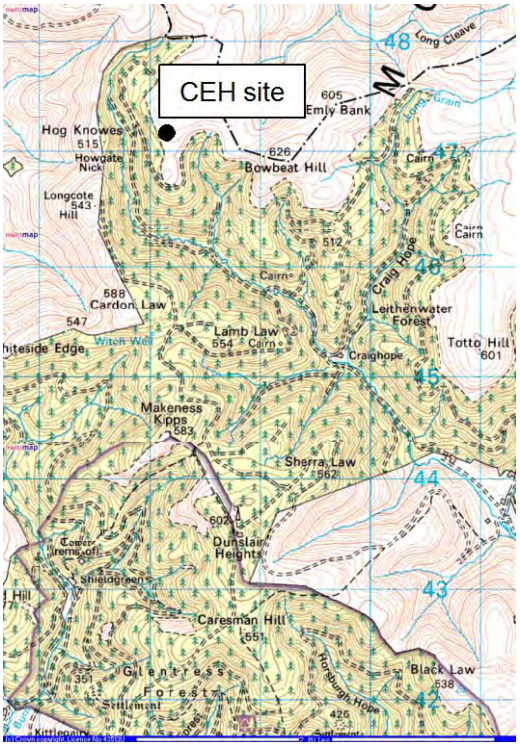

Figure 3.9. Map of Bowbeat Hill showing the location of the CEH monitoring site, the location for the vegetation sampling for this current project.

3.5.2. Botanical description

Bowbeat Hill is a typical blanket bog site (NVC; M19) dominated by *Calluna vulgaris* with locally frequent *Eriophorum vaginatum*, crowberry (*Empetrum nigrum*), and bilberry (*Vaccinium myrtilis*) and pleurocarpous mosses, especially *Pleurozium schreberi*. Evidence of its altitude (584 m above sea-level) is provided by the occasional presence of cloudberry (*Rubus chamaemorus*). Some areas show evidence of excessive burning for grouse rearing, where *Calluna* is virtually absent and cover is dominated by *Deschampsia flexuosa*, with locally abundant *Eriophorum vaginatum*, *Carex nigra*, *V. myrtilis* and *Galium saxatile*. Bryophyte cover is dominated by *Polytrichum commune* and *Rhytidiadelphus squarrosus*.

Figure 3.10. Photograph of Bowbeat Hill with the CEH monitoring station in the background above tree line and some of the wind turbines.

3.5.3. Summary of NH_3 concentration

The ammonia air concentrations measured during the experiment of this study are shown in Table 3.7. The concentrations at this site were rather low, but typical for such an upland location.

Table 3.7. Ammonia concentrations (μg m^{-3}) at Bowbeat Hill from 15-05-2004 until 17-07-2004.

Bowbeat Hill	15/05 – 16/06	16/06 – 17/07	Average
	0.47	0.31	0.39

3.5.4. Summary of N deposition

The orographic enhancement of wet deposition dominates the nitrogen inputs at this site. Without the orographic enhancement, N deposition at Bowbeat would be estimated at 8.5 kg N ha^{-1} y^{-1}. By contrast, inclusion of the orographic enhancement increases the estimated N deposition to approximately 25 kg N ha^{-1} y^{-1} (Pitcairn *et al*. In preparation).

3.6. Whim Moss

3.6.1. Site Description

Whim Moss (NGR: NT203532: altitude 280 m above sea-level) is a CEH Edinburgh experimental field facility in the Scottish Borders (Figure 3.7), which was established in 2002 as part the NERC GANE Programme to determine the effects of different forms of N on mire vegetation. The current experimental programme at Whim Moss is part of the Defra Terrestrial Acidification and Eutrophication Umbrella. The automated field release facility exposes the mire vegetation to either a) enhanced dry N deposition as gaseous NH_3 applied from a line source along a 60 m transect or b) wet N deposition applied as either NH_4^+ or NO_3^- via the enrichment of rainfall collected on site (Figure 3.11).

NH_3 release

Ammonia is released from a computer controlled 10 m line source, dispersing and diluting along a 60 m transect downwind (s/ssw to n/nne). The release rate is dependent on the prevailing weather conditions, with NH_3 being released when the wind direction is in the sector 180^0-215^0 and is > 2.5 m s^{-1} resulting in NH_3 fumigation being applied for between 3-10% of each monthly period. The NH_3 concentration is measured along the transect at 2 m, 4 m, 6 m, 8 m, 32 m, 60 m, 60 m/12 m east from the line source and at background ambient by a combination of passive samplers (NH_3 Diffusion tubes at 2-8 m and ALPHA samplers 8-60 m & ambient) at 0.1 m and 0.5 m above the height of the vegetation. The passive samplers are exposed for approximately 30 days. A full description of the automated NH_3 and wet N deposition field release systems is given by Leith *et al.* 2004.

Wet N treatments

The wet N treatments release system is fully automated and is dependent on sufficient precipitation volume and a wind speed of < 5 m s^{-1} to be activated. In the main Defra study there are eleven treatments, which are applied at four N deposition (control (10 kg N ha^{-1} y^{-1}), 16, 32 and 64 kg N ha^{-1} y^{-1}) and using oxidised and reduced forms of N ($NaNO_3$ – NH_4 Cl). In addition, there are $NaNO_3$ and NH_4Cl treatments at N deposition of 16 and 64 kg N ha^{-1} y^{-1} with added potassium and phosphorus, but these were not used in the current biomonitoring study. There are four randomised replicated 12.5 m^2 plots per wet N treatment (11 treatments x 4 replicates). In the biomonitors study only four of the treatments were used (control, 32 and 64 kg N ha^{-1} y^{-1}) with both forms of N and three replicate plots per treatment were used (see Table 3.8). Figure 3.11 shows the distribution of the plots in the experiment. Due to the short-

term exposure period (12 weeks) and the potentially small difference in N deposition between the 16 and 32 kg N ha^{-1} y^{-1} treatments, the former was not included in the biomonitors study.

Precipitation is collected on a 178 m^2 roof and stored in a 1200 litre tank. The individual stock solutions of the eleven treatments are added using individual treatment dozmatics to the collected precipitation. The treatments solutions (under a pressure of 1-2 Bar) are transferred along individual 16 mm tubing to each of the 44 treatment plots and applied to the plot by a 360^0 sprayer mounted on a central post.

As the application of both the wet and dry N treatments is solely weather dependent the frequency of treatment application mimics the 'real world' and therefore varies greatly between months. A full description of the automated wet N deposition field release system is given in Sheppard *et al*. (2004).

Table 3.8. Wet N deposition treatments (including background deposition) used in the *Deschampsia flexuosa* standardised grass study

Treatment	N deposition (kg N ha^{-1} y^{-1})	No of replicate plots per treatment
Control	10	3
$NaNO_3$	32	3
NH_4Cl	32	3
$NaNO_3$	64	3
NH_4Cl	64	3

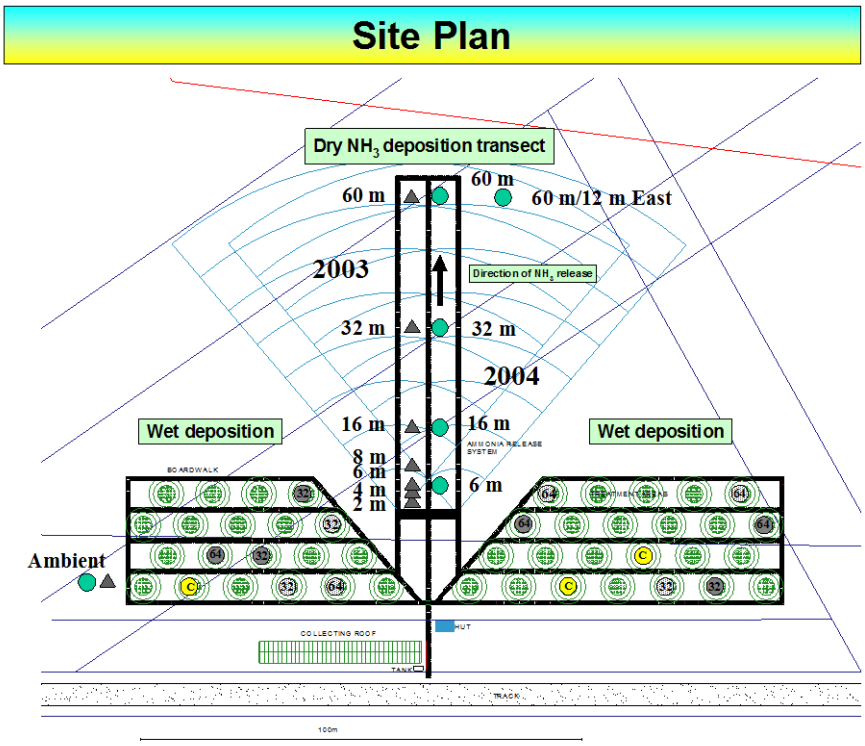

Figure 3.11. Schematic diagram of the field facility at Whim Moss showing the wet (2004) and dry N experimental treatments (2003 & 2004) as part of the biomonitors methods study.

3.6.2. Botanical Description

Whim Moss is an unmanaged *Calluna vulgaris*/*Eriophorum vaginatum* dominated mire with a diverse range of other ericaceous shrubs, lichens and acrocarpous and pleurocarpous mosses (NVC classification M19 (Averis *et al.* 2004)). The degenerative stage *Calluna vulgaris* with its open canopy is interspersed with *Eriophorum vaginatum* and an understorey cover of moss and lichen species (Table 3.9).

Table 3.9. Species composition at Whim Moss.

Species	Species continued.
Aulocomnium palustre	*Leucobryum glaucum*
Calluna vulgaris	*Mylia taylori*
Cladonia arbuscula	*Mylia anomala*
Cladonia chlorophaea	*Odontoschisma sphagni*
Cladonia ciliate	*Plagiothecium undulatum*
Cladonia crispate	*Pleurozium schreberi*
Cladonia fimbriata	*Polytrichum commune*
Cladonia gracilis	*Polytrichum juniperinum*
Cladonia portentosa	*Polytrichum alpestre*
Cladonia rangiferina	*Racomitrium lanuginosum*
Cladonia squamosa	*Sphagnum capillifolium*
Dicranum scoparium	*Sphagnum magellanicum*
Empetrum nigrum	*Sphagnum papillosum*
Erica tetralix	*Sphagnum russowi*
Eriophorum vaginatum	*Sphagnum fallax*
Hypnum jutlandicum	*Vaccinium oxycoccus*
Hypogymnia physodes	*Vaccinium myrtilus*

3.6.3. Summary of NH_3 concentration

The ammonia concentrations at Whim Moss for May-June 2003 and May-July 2004 are shown in Figures 3.12 and 3.13. In both years the NH_3 air concentrations were seen to decrease with distance away from the NH_3 line source.

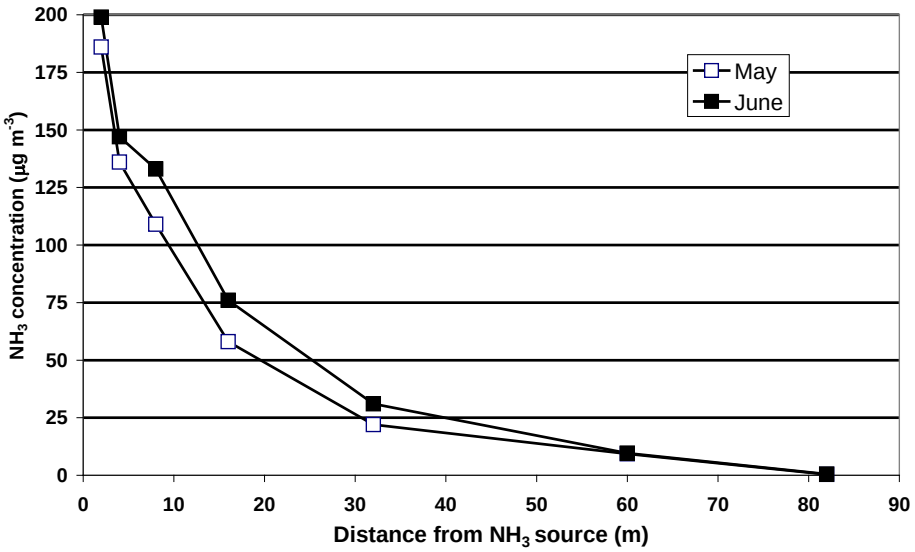

Figure 3.12. Whim Moss monthly mean atmospheric NH_3 concentration data for May and June 2003 along the NH_3 transect.

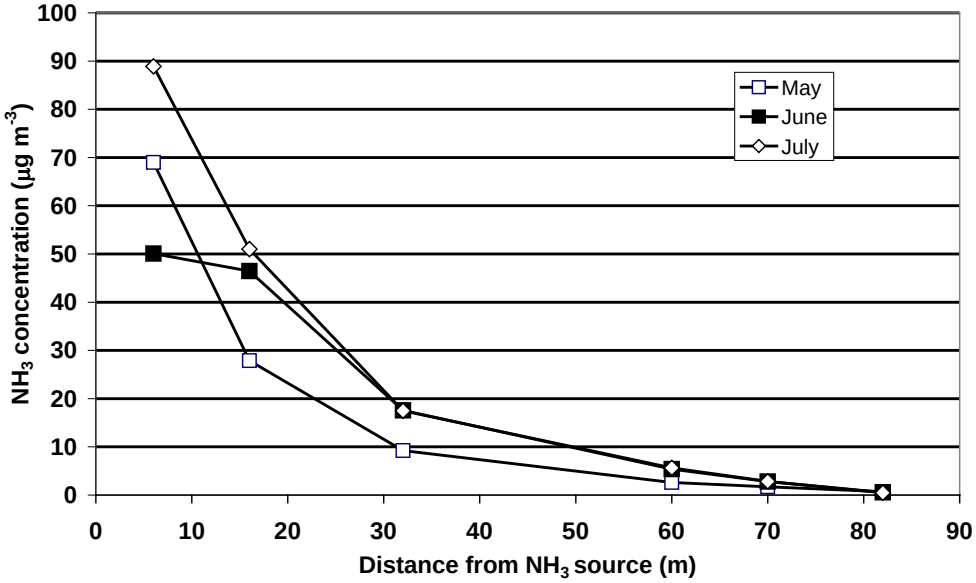

Figure 3.13. Whim Moss monthly mean atmospheric NH_3 concentration data for May, June and July 2004 along the NH_3 transect.

3.6.4. Summary of N deposition

The N deposition at Whim Moss has been measured continuously from May 2002. The total N deposition in Table 3.10 includes NH_3 deposition from monthly concentration measurements, wet N deposition and estimated nitric acid deposition.

Table 3.10. The total N deposition estimates for distances along the 60 m NH_3 transect at Whim Moss. (Values included background atmospheric N deposition).

Distance from NH_3 source	2 m	4 m	6 m	16 m	32 m	60 m	60 m /12 m East	Ambient
N deposition (kg N ha^{-1} y^{-1})	155	171	174	135	60	21	14.5	10

4. Refining and testing the Ellenberg Index biomonitoring method at the intensive sites

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4.1. Introduction

Ellenberg devised a comprehensive indicator system for vascular plants of central Europe (Ellenberg 1979; Ellenberg *et al.* 1992) to describe the response of individual species to a range of ecological conditions (light, temperature, continentality, moisture, pH and nitrogen). In several European studies, calculation of the Ellenberg Index of one or more variables for a given site has been used to indicate vegetation shifts due to land use changes and to increased atmospheric pollution (acidity and N deposition), by comparing plots or by following change in a single plot over time (e.g. Ellenberg 1988; Tyler 1987; van Dobben 1993).

In an earlier review of published studies on Ellenberg Indicators, Sutton *et al*. (2004a) concluded that in general, Ellenberg N values are useful tools in detecting floristic shifts consistent with increased nutrient availability and ecosystem eutrophication. The current aim is to test the suitability of the Ellenberg N Index for assessing the effects of N on condition and integrity of sites of conservation interest and to assess the advantage of this method over the critical loads approach. In addition, the robustness of the method and ease of application by agency staff will be discussed. While some sites may be close to obvious sources of N pollution, others may be subject to long-term increases in background N pollution. In this study, Ellenberg N Index was determined in four intensive study sites (Section 3), which include two sites adjacent to a gaseous N pollution source (vehicular NO_x and agricultural NH_3) and 2 sites with similar habitats, but different inputs of wet deposited N.

In arriving at a mean Ellenberg N Index for a sampling location, an N score is allocated to each plant species, so that the overall community has a score on a scale of nutrient poor (1) to nutrient rich (10). The index has been used at a local scale (e.g. Pitcairn *et al*. 2002; Falkengren-Grerup 1995; van Dobben 1993) and also at a national scale (Haines-Young *et al*. 2000). While most studies have focused on higher plants, the approach has been extended to cover bryophytes (van Dobben 1993; Pitcairn *et al*. 2002) using indicator values from Siebel (1993).

4.2. Methods

Ellenberg nitrogen indicator values at Piddles Wood SSSI and Happendon Wood were recorded at a range of points downwind of the expected pollution source, corresponding to the pollution monitoring sites (for site diagrams see Section 3). At Bowbeat Hill and Auchencorth Moss, in the absence of a pollution gradient within the sites, values were determined in two areas representative of the vegetation type in the vicinity of the NH_3 and precipitation monitoring equipment (see Table 4.1). Indices were determined using the modified values for British vascular plants (Hill *et al*. 1999) and indicator values from Siebel (1993) for bryophytes. The field process involved defining an area and recording all species readily observed in that area. The species were then allocated the appropriate indicator value and a mean indicator value for N was obtained. This value, based entirely on the presence of species, is termed an unweighted value. Weighted values may also be determined by measuring the abundance (% cover) of each species recorded. This measure necessitates

more extensive recording to arrive at a mean cover value for each species. Because of budget limitations, cover was limited to recording in an average of 5, 2 x 2 m quadrats at each point.

Table 4.1. Ellenberg surveys of four intensive Sites 2004.

Site	Date of survey	Methods used
Piddles Wood (SSSI adjacent to a poultry farms in Dorset)	23-24/06/04	The vegetation was surveyed in strips roughly 100 m long and 5 m wide at right angles to the ammonia monitoring transect at 6 distances from the poultry building, 5 sites corresponding to the NH_3 monitoring sites (10, 20, 40, 100 and 250 m from poultry unit) and an additional site at 70 m from the poultry unit. All species present were recorded. Percentage species cover was recorded in 3-6, 2 m x 2 m quadrats in each strip to give a rough mean percentage cover per strip for most species. Cover was recorded at 5 distances, 4 at NH_3 locations (10, 20, 40, 100 m) and also at 70 m.
Happendon Wood (Adjacent to M74 in south west Scotland)	16/06/04	The vegetation was surveyed in strips roughly 20 m long and 5 m wide at different distances from the M74 and at right angles to the ammonia monitoring transect. Six transects were surveyed, corresponding to monitoring sites 1-6. Transect 1 was not a continuous transect but a composite of 2 m x 2 m quadrats from more open areas. All species present were recorded. Percentage species cover was recorded in 3-6, 2 m x 2 m quadrats in each strip to give a rough mean percentage cover per strip for most species.
Bowbeat Hill (Upland moorland/blanket bog site near Peebles in southern Scotland)	17/06/04	At this site the vegetation is occasionally burnt for grouse shooting purposes and is hence composed of areas of *Calluna* moorland and grass-dominated moorland. The vegetation was surveyed in an area approximately 10 m x 10 m in both areas. All species present were recorded. Percentage species cover was recorded in 5 2 m x 2 m quadrats in each area to give a rough mean percentage cover for most species.
Auchencorth Moss (lowland moorland/blanket bog site near Penicuik in southern Scotland)	02/07/04	At this site, the ammonia is monitored inside the CEH atmospheric monitoring enclosure, fenced to exclude grazing animals. The vegetation was surveyed in an area approximately 10 m x 10 m both inside and outside the enclosure. All species present were recorded. Percentage species cover was recorded in 5, 2 m x 2 m quadrats in each area to give a rough mean percentage cover for most species.

4.3. Results

4.3.1. Piddles Wood SSSI

Piddles Wood is oak/hazel woodland with a rich ground flora (see Section 3.2.2. for a full botanical description). The study area which stretches southwards from the poultry unit downhill for 250 m, although less rich than some areas of the SSSI, yielded a total of 82 species (vascular plants + bryophytes), ranging from 29 species close to the poultry unit to 44 species 50-100 m downwind of the poultry unit . The mean Ellenberg N Indicator at Piddles Wood declined from 5.9 at 10 m from the poultry unit to 5.2 at 100 m and 4.8 at 250 m downwind of the poultry unit (Figure 4.1a). Standard deviations were large but the linear regression of the trend against log distance was significant (R^2=0.89). The mean cover weighted Ellenberg index showed a steeper decline with distance from the poultry unit and the trend against log distance was also significant (R^2= 0.71) (Figure 4.1b). When plotted against log mean ammonia concentrations for the study period (April-July 2004), the trends remained significant (R^2 =0.64) suggesting that ammonia emissions from the poultry unit have had a major influence on species composition of the woodland (Figure 4.2a and 4.2b). Similar trends were obtained between mean total estimated atmospheric N deposition for the study period, and both unweighted (R^2 =0.75) and cover weighted (R^2 =0.80) Ellenberg N indices (Figure 4.3a and 4.3b).

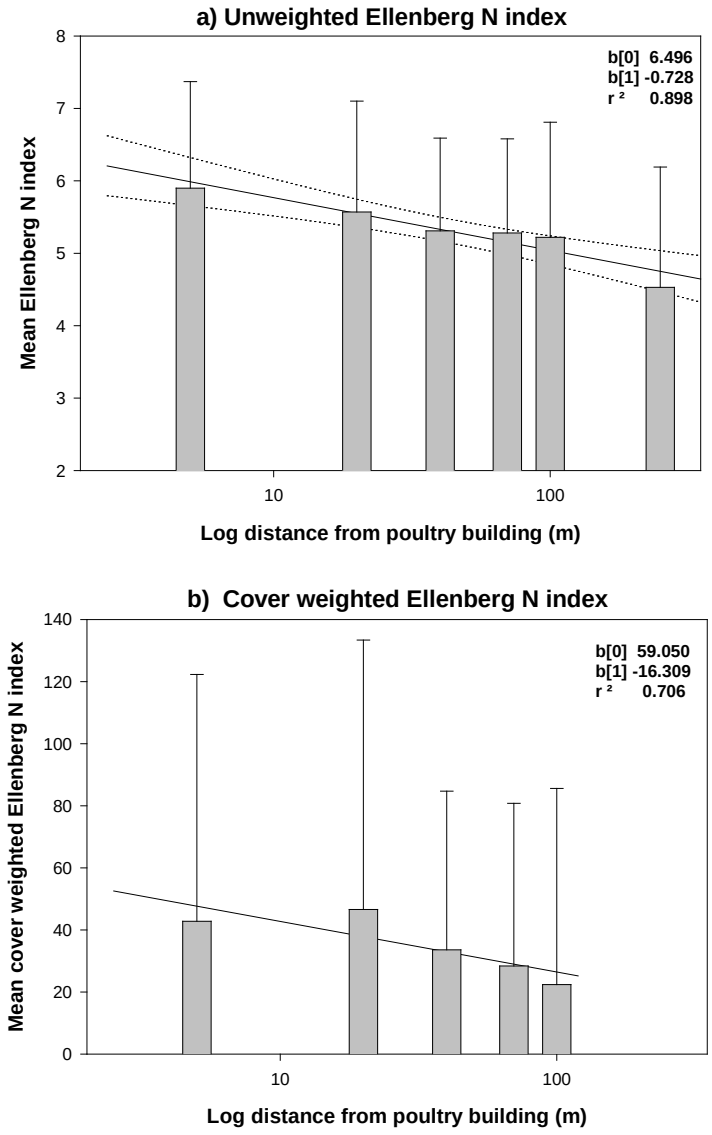


Figure 4.1. Relationship between Ellenberg N Index a) unweighted and b) cover weighted and log distance downwind of a poultry unit at Piddles Wood, Dorset. Cover estimates were not made at a distance of 250 m owing unsuitable terrain.

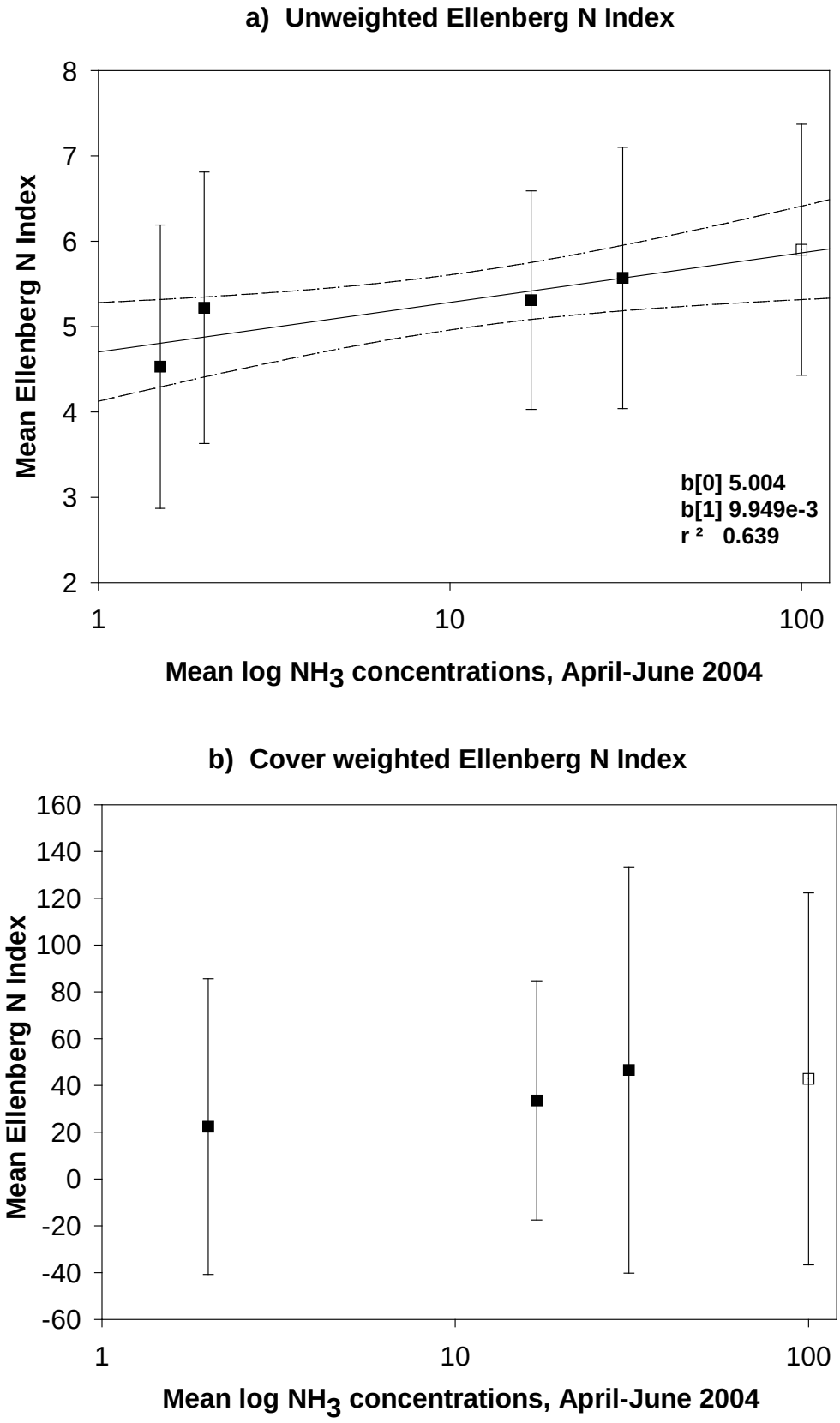


Figure 4.2. Relationship between mean Ellenberg N index, a) unweighted and b) cover weighted, and mean ammonia concentration, downwind of a poultry unit at Piddles Farm, Dorset. NH_3 was not monitored at a distance of 70 m, reducing the number of points on the graphs to 5 in a) and to 4 in b).

At the top of the slope close to the poultry unit, cover was dominated by ivy (*Hedera helix*), with chervil, (*Chaerophyllum temulentum*), yellow archangel (*Lamiastrum galeobdolon*),

bluebell (*Hyacinthoides non-scripta*) and nettle being locally frequent. In many of the species present, the leaves were exceptionally large. Many of the trees had moss cover, but vigour was poor due to overlying layers of algae. Further down the slope at 20 m from the poultry unit, bluebell was locally abundant and *Poa trivialis,* dog's mercury (*Mercurialis perennis*) and enchanter's nightshade (*Circea lutetiana*) were locally frequent. *Dryopteris dilatata* was recorded and bryophyte species were more numerous.

At 40-100 m downwind of the poultry unit, dog's mercury and enchanters nightshade were locally abundant, 4 species of fern were found and 18 species of bryophyte. Vernal species, such as primrose (*Primula vulgaris*) and wood anemone (*Anemone nemoralis*), which die back by May, were only occasionally recorded. *Carex sylvatica* was locally frequent and occasional specimens of *Dactylorhiza fuchsii* and *Conopodium majus* were observed. At around 100 m from the poultry unit, areas of open canopy were dominated by bracken (*Pteridium aquilinum*) and bramble (*Rubus fructicosus*). Canopy conditions at the site 250 m downwind, were considerably different and species were recorded largely on the woodland edges. Dominant species included grasses, pleurocarpous mosses and *Polytrichum* spp.

While some of the changes in species composition with distance from the poultry unit could be influenced by underlying soil type (pH and drainage, although this was not recorded) and canopy cover, there is undoubtedly a trend from predominantly nitrogen loving species towards a richer flora of less N loving species with increasing distance from the poultry unit. The Ellenberg N Index reflects this trend.

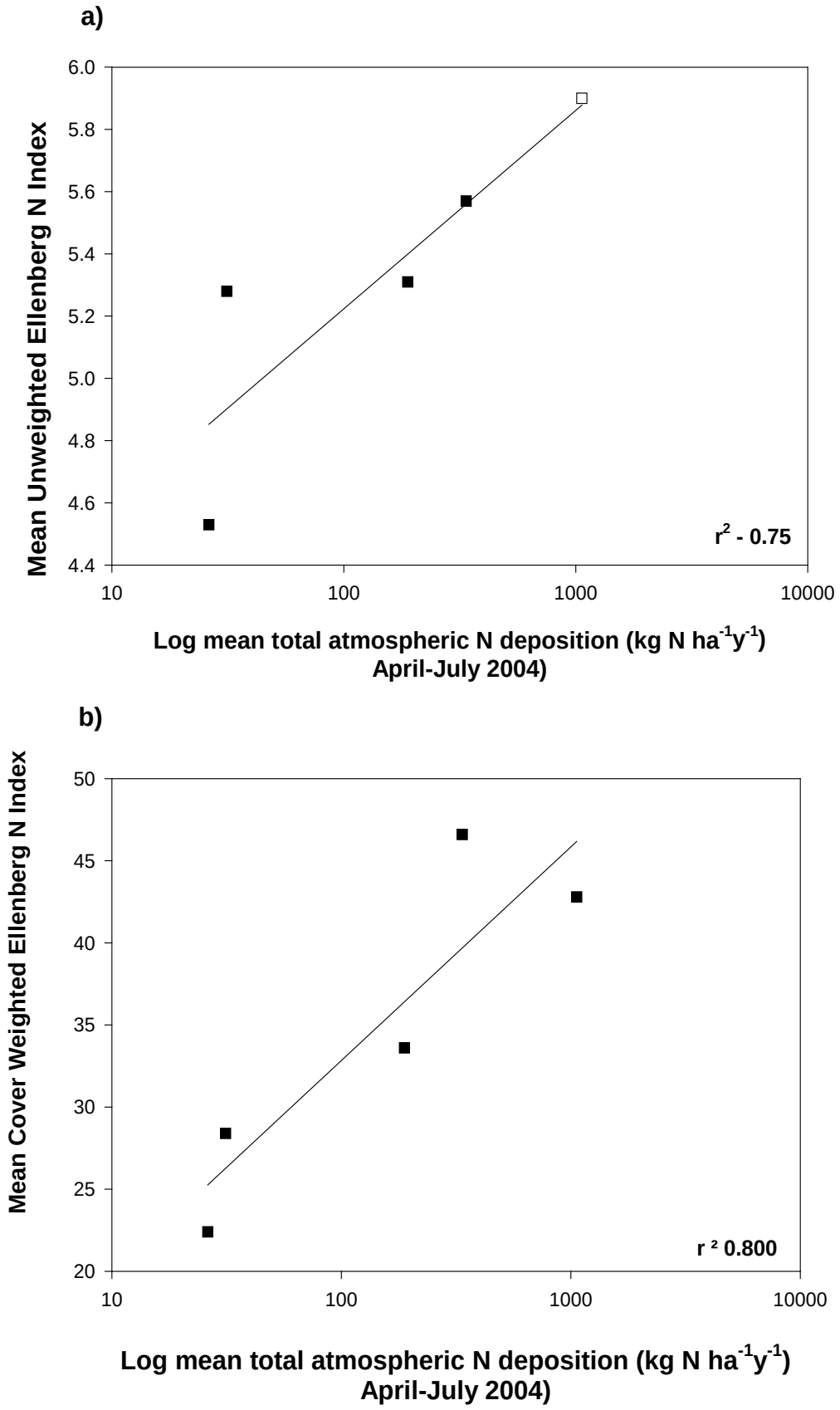


Figure 4.3. Relationship between total atmospheric N deposition and mean Ellenberg N Index, a) unweighted and b) cover weighted, downwind of a poultry unit at Piddles Wood, Dorset. (Details of the atmospheric N deposition estimates and their uncertainties are provided in Section 3).

Comparisons with earlier records

Details of Nature Conservancy Council Surveys of the woodland made in 1985 and 1991 were provided by English Nature. Although the records were specific to large areas, some broad comparisons have been made with current 2004 records as shown in Table 4.2.

Table 4.2. Comparisons of vegetation surveys made in 1985 and 1991 with the 2004 survey.

Year	1985	1991	2004
0-70 m from poultry unit	*Luzula sylvatica/ Carex sylvatica* - D. *Hedera helix , Mercurialis perennis* - F	*Mercurialis perennis* - D *Luzula sylvatica, Hedera helix* – A *Galium aparine* - F	*Hyacinthoides non-scripta, Hedera helix* – A *Holcus mollis, Lamiastrum galeobdolon, Glechoma hederacea, Mercurialis perennis, Circea lutetiana, Urtica dioica, Dryopteris dilatata*- LF
100 m from poultry unit	Dense hazel coppice: *Pteridium aquilinum* - A: *Hyacinthoides non-scripta , Holcus mollis* - F; *Lonicera periclymenum* - O	Hazel cut in 1990: *Pteridium aquilinum /Rubus fructicosus* - A; *Luzula sylvatica, Holcus mollis* - F Uncut hazel: *Holcus mollis* - D *Luzula sylvatica, Melica uniflora, Pteridium aquilinum* - LF	*Pteridium aquilinum* – A *Mercurialis perennis, Carex sylvatica, Oxalis acetosella, Urtica dioca, Veronica chamaedrys* - F

The 1985 and 1991 surveys were both conducted prior to the establishment of the poultry unit in 1993. The comparison therefore shows the potential impacts of the poultry unit on the woodland during the last 10 years. Since 1985, *Luzula sylvatica* (NI-4), *Carex sylvatica* (NI-5) and *Mercurialis perennis* (NI-7) have declined close to the poultry unit and have been replaced by *Lamiastrum galeobdolon* (NI-6), *Glechoma hederacea* (NI-7). Although these changes indicate a move towards more N-loving species, the impact of differing light levels resulting from coppicing cannot be ruled out. The latter 2 species, which are shade tolerant and hence present in the field layer prior to coppicing, are able to respond to increased light levels following coppicing, spreading rapidly by means of creeping shoots.

4.3.2. Happendon Wood

As described in Section 3.4, Happendon Wood is a split site, one part comprising mixed conifer woodland along the M74 motorway and the other part comprises a mature Beech/oak Woodland some 200 m from the M74. The woodland is not continuous, and between the 2 parts runs the B7078. The survey transect was species rich with 78 species (vascular and bryophytes) recorded along the transect from the M74 into the beech woodland. The mean unweighted Ellenberg N index ranged from 5.7 at 10 m, 5.2 at 20 m., 4.9 at 38 m., 4.9 at 150 m, and 4.2 at 200m and 250 m from the M74. The site at 38 m was largely unshaded, and included a wider range of species, which tended to lower the mean N index. However despite differences in woodland type and canopy cover, the relationship between the N index and log distance was significant (R^2 =0.85) (Figure 4.4).

The correlations were somewhat lower when mean Ellenberg score was plotted against the nitrogen parameters, with R^2 values of 0.71, 0.55 and 0.66 against *ln* values of NO_2, NH_3 and N deposition, respectively. This may be explained by the unrepresentative nature of site 3, which had significantly higher NH_3 and NO_x concentrations than would be expected based on its distance from the M74. This was the site adjacent to the small waste water/ sewage plant, indicating that during the sampling period NO_x and NH_3 concentrations were influenced by this source. If the values from Site 3 are excluded, the correlations are much higher, with R^2 values of 0.96, 0.68 and 0.81 against *ln* values of NO_2, NH_3 and N deposition, respectively

(Table 4.3). The highest correlation is thus with NO_2 concentrations, which is consistent with this being the main cause of altered N deposition across the transect.

Table 4.3. Summary of the data and different correlation coefficients.

	Site	**M74**	**NH_3 µg m^{-3}**	**NO_x µg m^{-3}**	**N dep. kg N ha^{-1} y^{-1}**	**Ellen. N**
	1	10	1.3	13.4	22.8	5.7
	2	20	0.5	11.5	14.3	5.2
	3	38	0.9	13.9	19.4	4.9
	6	150	0.5	8.5	13.2	4.9
	4	200	0.5	6.3	11.7	4.2
	5	250	0.4	5.6	12.6	4.2
Data from all sites	Correlation with Ellenberg N value (R^2)	0.78	0.55	0.67	0.65	
	Correlation of *ln*(x) with Ellenberg N value (R^2)	0.84	0.55	0.71	0.66	
Excluding Data from Site 3	Correlation with Ellenberg N value (R^2)	0.88	0.63	0.97	0.76	
	Correlation of *ln* (x) with Ellenberg N value (R^2)	0.87	0.68	0.96	0.81	

Cover weighted mean Ellenberg N index showed no trend with distance, NO_2 concentrations or N deposition and error bars were very large.

Close to the M74 (10 m), the coniferous woodland canopy was fairly dense with patchy, floppy grass cover (*Holcus mollis, Festuca rubra*) and abundant bryophyte cover (mainly 4 species). At 20 m, species diversity increased considerably (from 15 to 34 spp.) including a 3-fold increase for bryophytes. Nitrogen loving species such as raspberry (*Rubus idaeus*), rose-bay willow herb (*Chamaenerion angustifolium*,) and nettle (*Urtica dioica*) were locally abundant in more open areas, together with bryophytes *Scleropodium purum* and *R. squarrosus.*

Figures 4.5 and 4.6 show the response of Ellenberg score to NO_x concentrations and N deposition respectively.

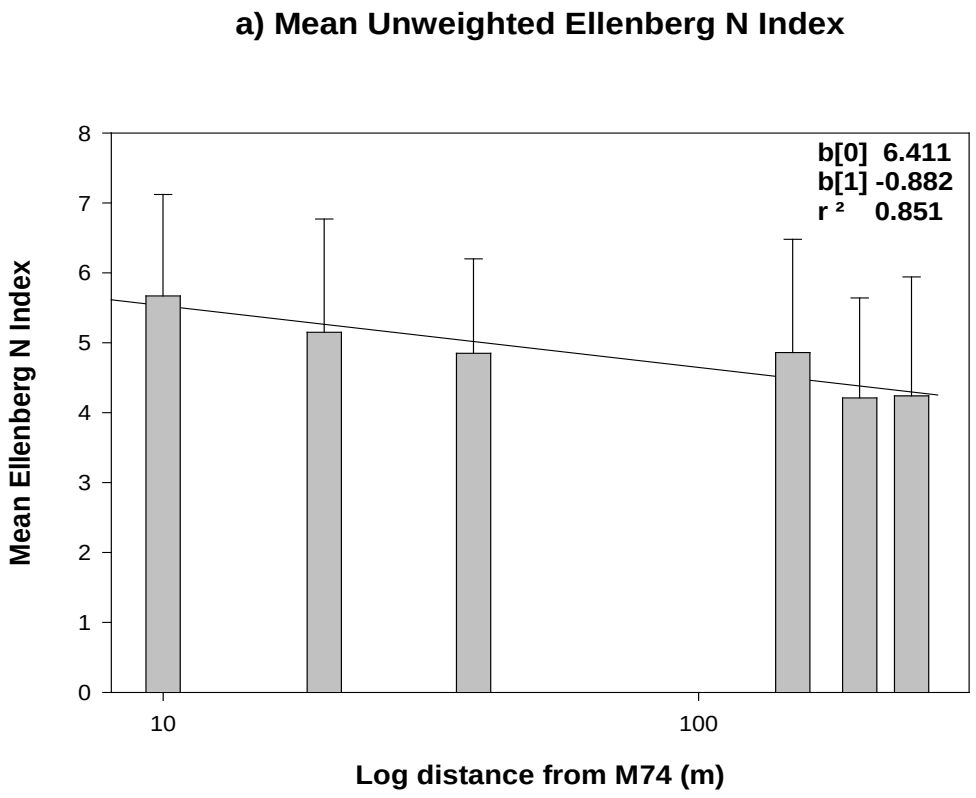


Figure 4.4. Relationship between mean unweighted Ellenberg N Index and log distance from the M74, Happendon Wood, south Lanarkshire.

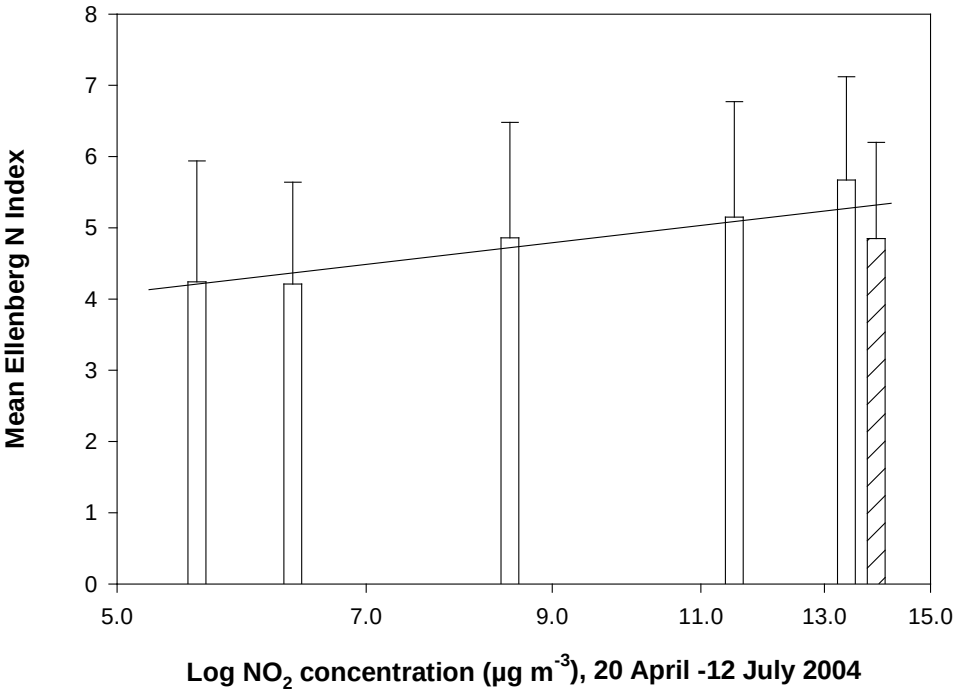


Figure 4.5. Relationship between mean unweighted Ellenberg N index and log NO_2 concentration downwind of the M74, Happendon Wood, south Lanarkshire. The hatched column represents site 3 (when all sites included, R^2 =0.714; when site 3 excluded, R^2 =0.960).

The mature beech woodland supported a limited ground flora due to fairly heavy leaf litter. *Holcus mollis* and *Deschampsia flexuosa* were locally abundant, and the most common bryophytes included *Rhytidiadelphus loreus, R. triquetrus, Dicranum scoparium* and *Polytrichum formosum*. Where the woodland borders on the B7078, *Galium aparine, Veronica chamaedrys* and *Viola riviniana we*re locally frequent and *Anemone nemorosa, Conopodium majus* and *Succisa pratensis* are occasional. The area most distant from the M74 has a mixed canopy of *Larix decidua, Betula pendula* and *Pinus sylvestris* and the soil is damper and appears to be more base rich. *Holcus mollis* and *Juncus effusus* were locally abundant, *Agrostis stolonifera, Anthoxanthum odoratum,* and *Molinia caerulea* were frequent and occasionals included *Lotus uliginosus, Lathyrus pratensis,* and *Lynchis flos-cuculi.*

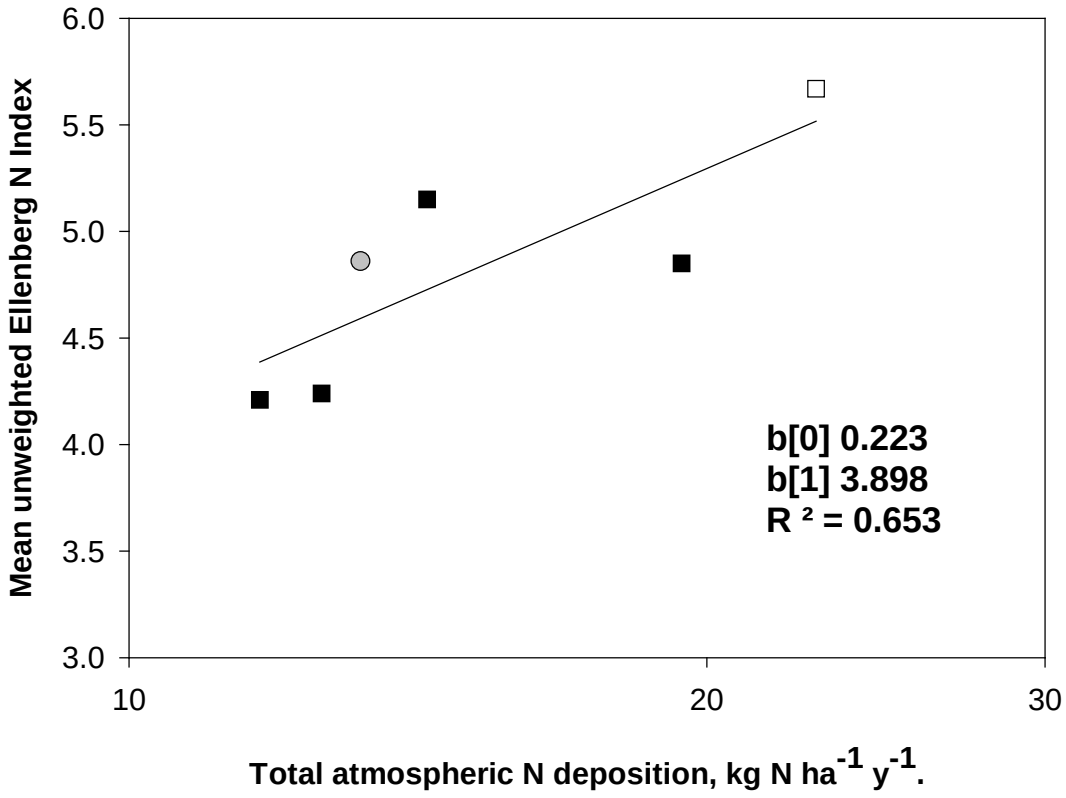


Figure 4.6. Relationship between mean unweighted Ellenberg N index and log total atmospheric N deposition (kg N ha^{-1} y^{-1}). The grey circle represents site 3.

4.3.3. Effects of enhanced wet deposition: Bowbeat Hill vs. Auchencorth Moss

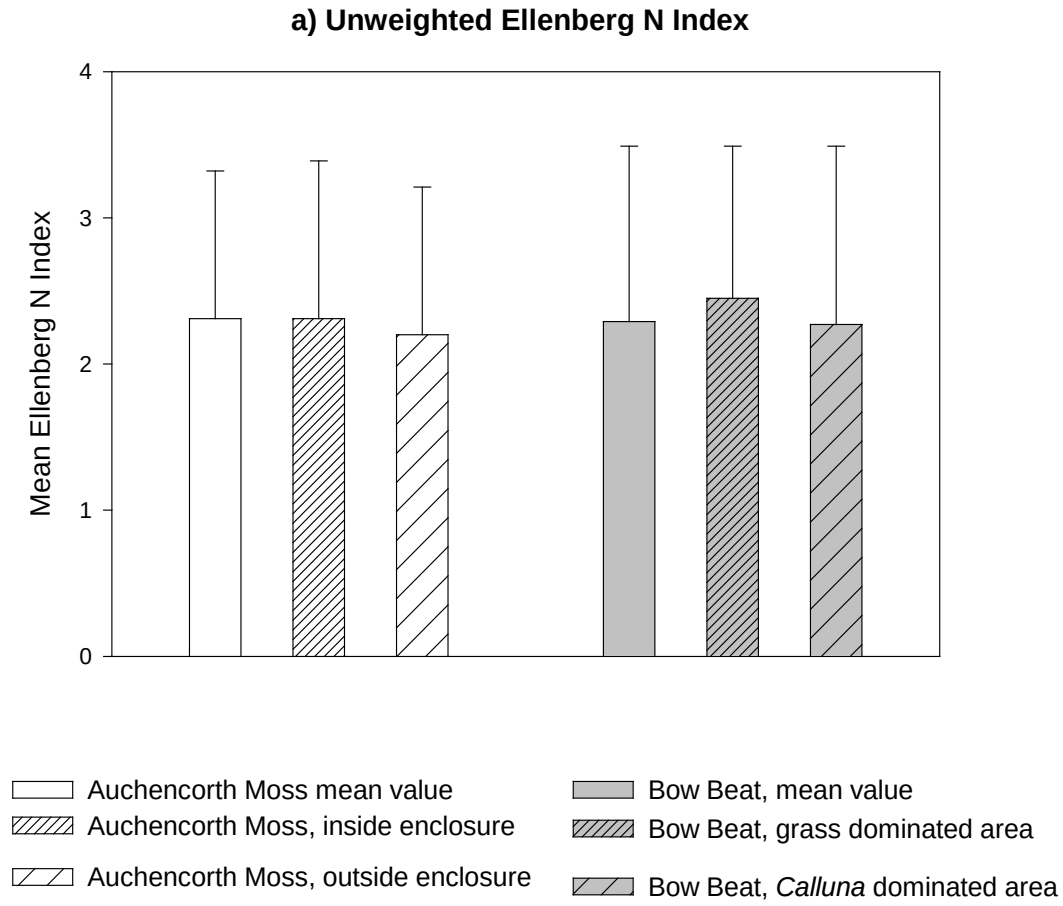
Bowbeat Hill is a typical blanket bog site with large areas dominated by dwarf shrubs and smaller areas dominated by grass species (due to management changes, possibly burning for grouse rearing). Surveys took place in both areas in July 2004. In the *Calluna* dominated areas, *Eriophorum vaginatum*, crowberry (*Empetrum nigrum*), and bilberry (*Vaccinium myrtilis*) were locally frequent and cloudberry (*Rubus chamaemorus*) was occasional. Pleurocarpous mosses, especially *Pleurozium schreberi* were common. In the grass dominated areas, *Calluna* was virtually absent and cover was dominated by *Deschampsia flexuosa,* with locally abundant *Eriophorum vaginatum, Carex nigra, V. myrtilis* and *Galium saxatile*. Bryophyte cover was dominated by *Polytrichum commune* and *Rhytidiadelphus squarrosus.* Unweighted and weighted Ellenberg N indicies were obtained for both *Calluna* and grass dominated areas. Figure 4.7 shows that the mean index is almost identical for both areas (NI-2.3) and indicates a site of low available N. The cover-weighted index was larger for the grass

dominated area reflecting the abundance of the more N loving (NI-5) *R. squarrosus* compared with the abundance of *Pleurozium schreberi* (NI- 2) in the *Calluna* dominated area.

The mean Ellenberg N index for the site is 2.3 despite the fact that the total modelled N deposition for the 5 x 5 km square is 25 kg N ha^{-1} y^{-1}, which includes seeder-feeder enhanced wet deposition. Auchencorth Moss is part of a large valley bog which has been extensively drained for peat extraction in areas. The presence of a CEH monitoring site within an enclosure allows recording in both ungrazed and grazed areas. In the grazed area, *Molinea caerulea* was dominant and *Eriophorum vaginatum, Deschampsia flexuosa* and *Juncus squarrosus* were locally abundant. *Calluna vulgaris* was absent but *Erica tetralix* occurred occasionally. Bryophyte cover included locally frequent *Hylocomium spendens, Pleurozium schreberi, Rhytidiadelphus squarrosus* and *Sphagnum capillifolium.*

Within the ungrazed enclosure, *Eriophorum vaginatum* and the bryophytes *Pleurozium schreberi* and *Polytrichum commune* were dominant. *Juncus squarrosus* was absent and *Galium saxatile* was locally frequent. The unweighted mean Ellenberg N index was 2.3 (2.3 outside the enclosure, 2.2 inside). Cover weighted Ellenberg N Index was slightly higher inside the enclosure reflecting the large bryophyte cover (Figure 4.7).

Both Auchencorth Moss (low N deposition) and Bowbeat Hill (larger N deposition) show similar values for the mean unweighted Ellenberg scores. However the cover weighted Ellenberg values showed a difference between and within sites. At Bowbeat, the cover weighted index for the grass dominated area was higher than that of the *Calluna* dominated area and that for Auchencorth. While the between site differences is consistent with the increased N wet deposition at Bowbeat Hill, the large difference between the two sample locations at Bowbeat Hill indicates that local variability in plant communities could have a large influence on the result.



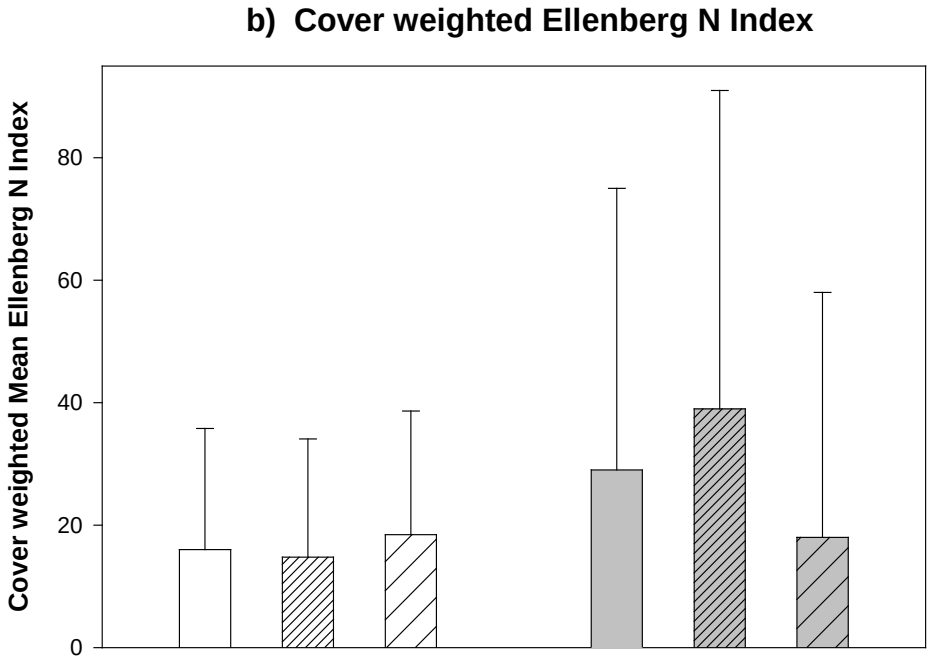


Figure 4.7. Mean Ellenberg N Index, a) unweighted and b) cover weighted for Bowbeat Hill and Auchencorth Moss.

4.4. N deposition

The mean Ellenberg Index (unweighted and cover-weighted) for all points recorded in the 4 intensive sites has been plotted against log total N deposition in Figure 4.8. The relationship is weak for the mean unweighted Index (R^2=0.26) but much stronger for the cover weighted Index (R^2=0.69). The relationship between cover weighted Ellenberg Index and N deposition is better than might be expected from such a disparate group of sites suggesting that the Ellenberg N Index can provide a useful indication of enhanced N deposition.

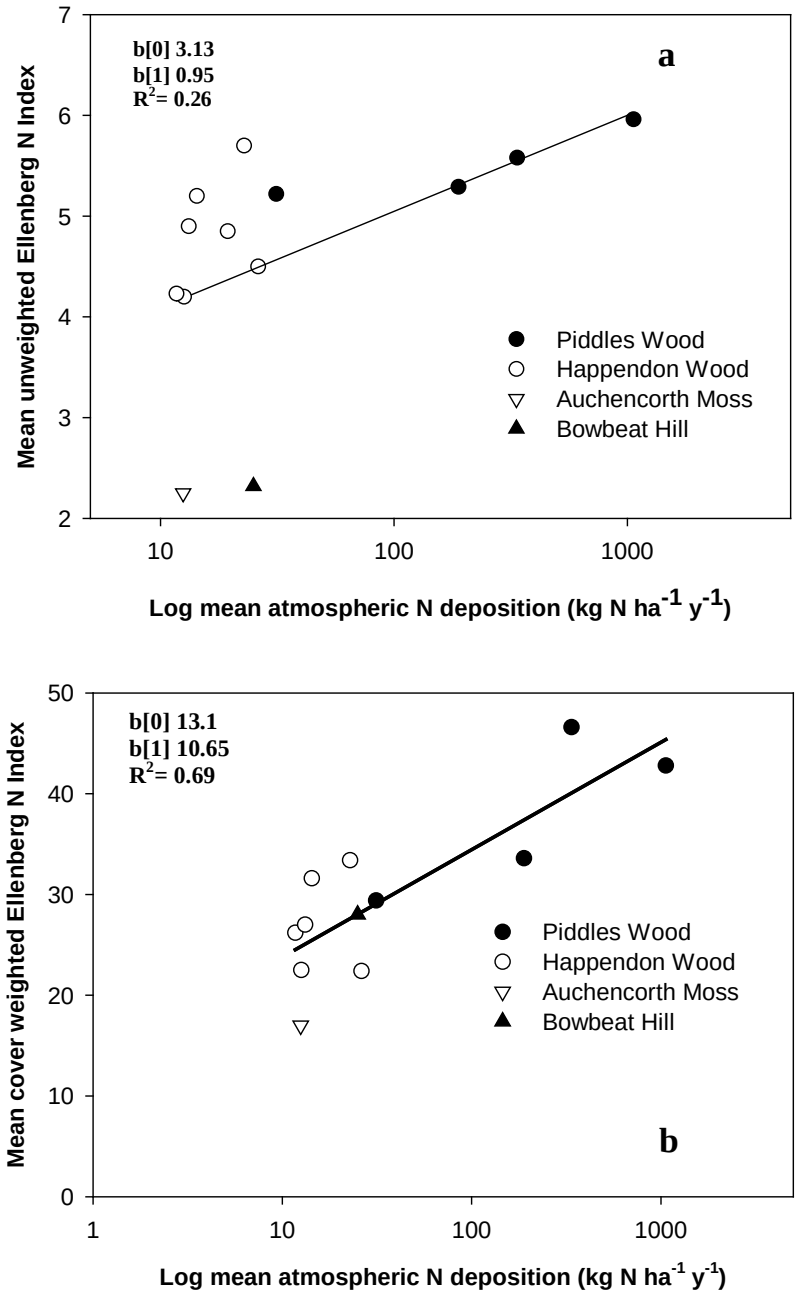


Figure 4.8. Relationship between Ellenberg N Index for all points recorded in the 4 intensive sites, and log total annual N deposition, for a) unweighted and b) cover weighted index.

4.5. Acidophyte/nitrophyte Index

Biomonitoring, using tree lichens to detect atmospheric N in agricultural areas, first in the Netherlands (van Herk 1999) and later in the UK (Wolseley and James 2002a), involved the development of indicies of nitrophyte and acidophyte lichens. Acidophytes prefer naturally acidic bark while nitrophytes prefer enriched, more basic bark, resulting from enhanced NH_3 concentrations. This approach was used very successfully at Earlston poultry farm in southern Scotland (Sutton *et al.* 2004a). Subtraction of the nitrophyte score from the acidophyte score provided an index showing whether the flora was dominated by acidophytic or nitrophytic species.

A first attempt has been made to apply this approach to the vascular plant and bryophyte species recorded at the 4 intensive sites, paying particular attention to Piddles Wood. Species have been described as acidophyte or nitrophyte mainly on the basis of their Ellenberg scores – e.g. those with scores of 4 and below were described as acidophytes and those with 6 and above, as nitrophytes. Species with an index of 5 (intermediate N plants) were excluded together with those species, which are not so easy to identify and those which can be readily overlooked in the field, such as liverworts. Species with scores of 6 or greater, but characteristic of the NVC class such as dogs mercury and bluebell were also excluded. Species with a low NI score, but known to respond to added N from studies in the UK, Netherlands and Sweden (Pitcairn *et al.* 1998) were described as nitrophytes because of their potential to out-compete small herbs in eutrophicated environments. These include the grasses *Deschampsia flexuosa, Festuca ovina* and *Molinia caerulea*. Although these species naturally occur as low index plants in many habitats their presence and perhaps more importantly, a significant cover of such species should be carefully noted.

In general, the acidophyte species tended to be vernal species, (necessitating early surveys), orchids, woodrushes and several bryophyte species typical of acid woodlands and moorland. Nitrophytes tended to be 'weed species' such as nettle, dock, hogweed, chervil, willow herbs and grasses typical of arable land together with a few bryophytes, which prefer N enriched habitats.

Results for Piddles Woods based on subtracting nitrophyte presence from acidophyte presence (Figure 4.9), showed that the flora is dominated by nitrophytes at mean concentrations of NH_3 above 3 $\mu g\ m^{-3}$ (the change occurs between the locations with concentrations 3 and 8 $\mu g\ m^{-3}$). When subtracting total nitrophyte cover from total acidophyte cover, the flora were found to be dominated by nitrophytes at NH_3 concentrations scarcely above 1 $\mu g\ m^{-3}$. However it must be pointed out that the Piddles Wood survey took place in July 2004 when only remnants of vernal species such as wood anemone were visible. In addition, changes in soil type, canopy cover and leaf litter levels would also affect cover.

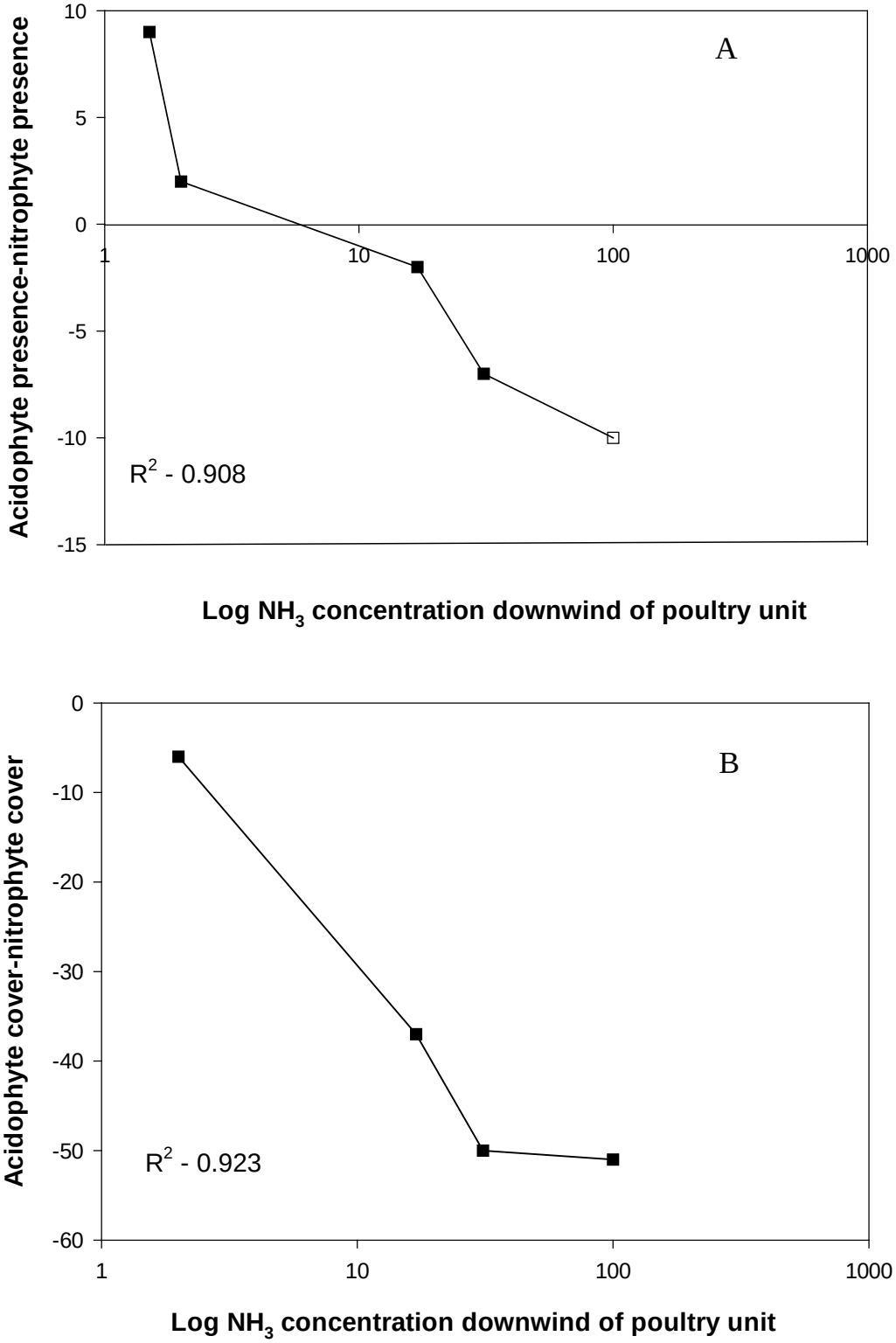


Figure 4.9. Eutrophication estimate for Piddles Wood, using Ellenberg N index data plotted against log NH_3 concentrations. a) acidophyte-nitrophyte presence, b) acidophyte-nitrophyte cover. NH_3 measurements are available for 5 sites; cover estimates are available for 4 sites.

At Happendon Wood the relationship between acidophyte-nitrophyte species for both presence and cover and NO_2 concentrations is very good (Figure. 4.10), nitrophytic species dominating the flora at NO_2 concentrations greater than 8 µg m^{-3}.

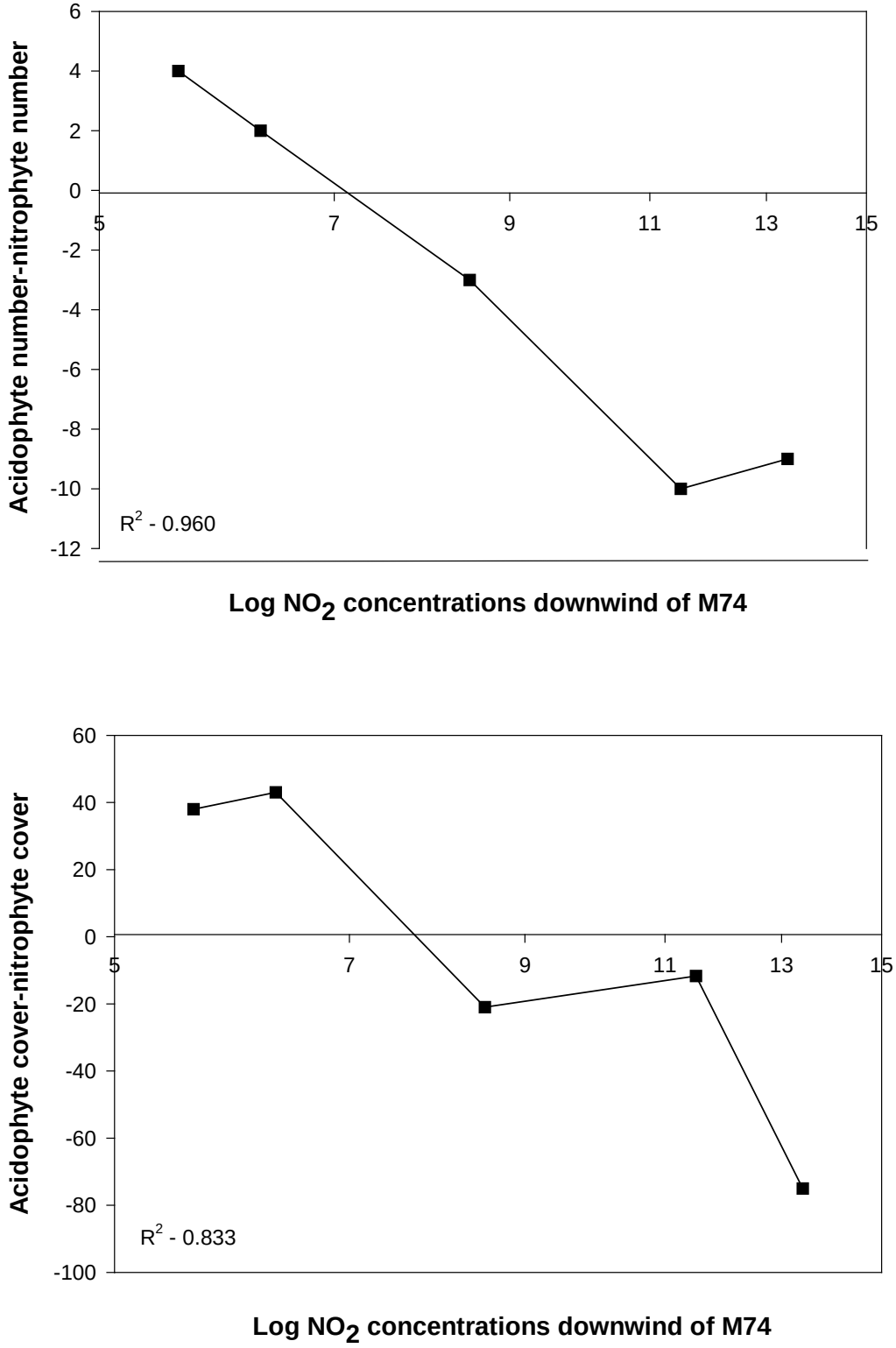
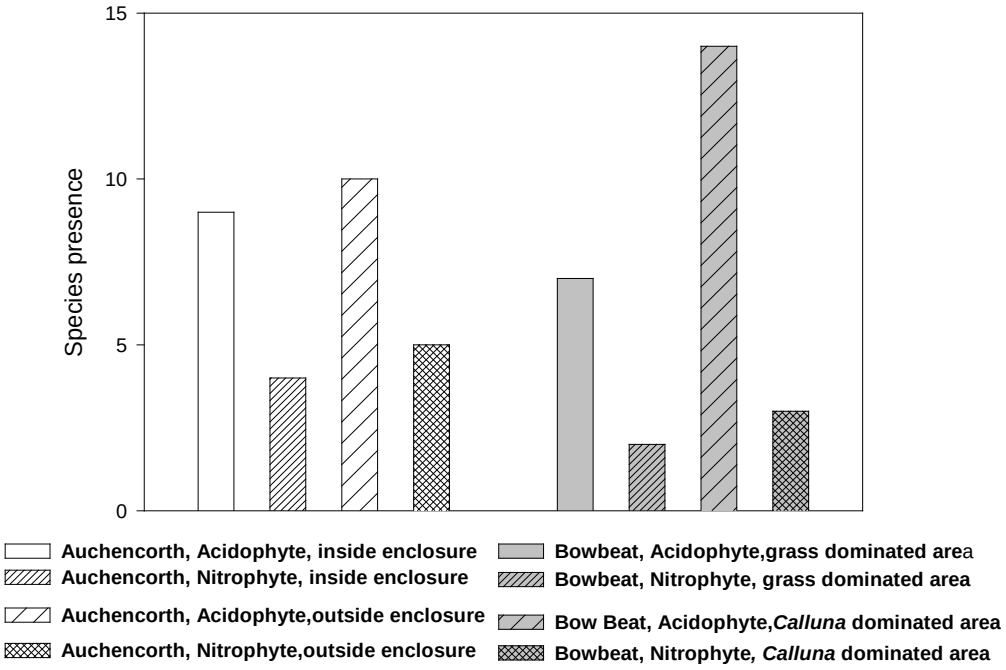


Figure 4.10. Eutrophication estimate for Happendon Wood, using Ellenberg N index data plotted against log NO_2 concentrations.

At Auchencorth Moss and Bowbeat Hill (Figure 4.11), similar results based on species presence were obtained, acidophytes dominating the vegetation. However when the cover of acidophytes and nitrophytes was examined, a rather different picture emerged. For example in the grass dominated area at Bowbeat Hill nitrophyte species although fewer in number than acidophytes, almost equalled the cover of acidophytes. This expansion of certain species in the absence of dwarf shrubs, suggests potential problems for species diversity in grassy areas of blanket bogs in high N input regions.



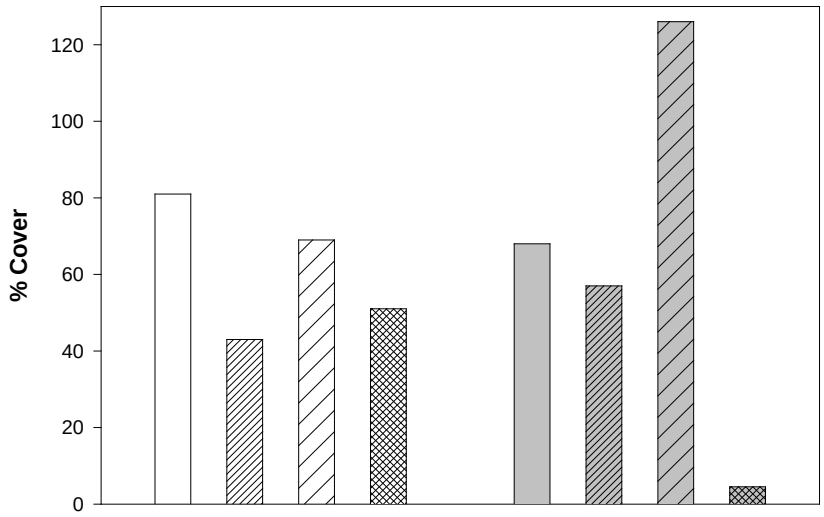


Figure 4.11. Acidophyte-nitrophyte presence and cover indices for Auchencorth Moss and Bowbeat Hill.

4.6. Discussion

4.6.1. Piddles Wood

At Piddles Wood SSSI, the Ellenberg N Index showed a significant decline with increasing distance from the poultry houses. There was also a significant linear regression between both the mean unweighted and weighted Ellenberg N Indicies and ammonia concentrations. The results are compatible with published data (Pitcairn *et al*., 2002, 2003) from the vicinity of livestock farms in southern Scotland. It is interesting to compare the actual Index values for Piddles Wood, a managed oak wood, and Earlston, (an extensively researched site in Scotland, where pilot studies took place in the first phase of this project, Sutton *et al*. 2004a), which is mixed woodland with areas of conifer plantation. At Earlston, annual mean ammonia concentrations declined from 29 µg m^{-3} at the woodland edge, 15 m from the poultry houses, (Pitcairn *et al*. 1998) to 1.6 µg m^{-3} at 276 m, and total nitrogen deposition at the woodland boundary was estimated at around 190 kg N ha^{-1} y^{-1} (Sutton *et al*. 2004a). At 15 m downwind where species change was visible, the Ellenberg Index was 4.9 declining to 3.9 at 276 m.

At Piddles Wood over the 12-week study period, mean ammonia concentrations at a comparable distance from the poultry house (20 m) were similar (31 ug m^{-3}) to those at the Earlston Farm and total atmospheric N deposition was estimated to be 337 kg ha^{-1} y^{-1}. The Ellenberg Index declined from 5.2 at 20 m to 4.2 at 250 m, not unlike those found at Earlston, but with N Index values approximately 7% larger. Cover weighted Index values showed a better relationship with NH_3 concentrations at Piddles Wood compared with the Earlston farm transect, again probably due to the large abundance of low Index grass species close to the poultry house at Earlston.

Spatial impacts on species composition at both Piddles Wood and Earlston, based on species presence, were mainly detected in the first 50 m although the cover weighted index at Piddles Wood suggested an impact for the first 100 m at least.

Temporal impacts on species composition at Piddles Wood using data from past surveys suggest a move to a more nitrophytic flora since the establishment of the poultry farm, indicating a probable impact of N emissions from the farm on species composition.

Acidophyte/Nitrophyte Index: First attempts to develop an index based on two broad groups of N preference have provided useful information on the eutrophication status of the site. Nitrophytic species were found to dominate the flora at NH_3 concentrations greater than 3 µg m^{-3}. However, the allocation of preference to individual species may not always be suitable, as soil type, canopy cover and litter levels may affect cover.

Overall, the first test of this acidophyte/nitrophyte approach for higher plants and bryophytes indicates a high sensitivity compared with the classical Ellenberg approaches. This is to be expected, since the approach focuses on the species which are most likely to change in response to altered N supply, excluding those which are less sensitive to nitrogen. Similarly, the species denoted as acidophytes and nitrophytes may need to be defined on a habitat specific basis according to the results of experimentation on N deposition responses. Clearly the next stage should be the development and refinement of lists of key species of acidophytes and nitrophytes for major habitats. The process should be started by expanding the data base currently set up for woodland habitats, followed by evaluation using a wide range of sites in the UK.

4.6.2. Happendon Wood

The Ellenberg Index also proved reasonably successful in detecting the influence of road emissions from the M74 on woodland groundflora downwind, despite lack of homogeneity in canopy cover and woodland type. The N index declined from 5.8, at 10 m from the M74 to 4.2 at 250 m downwind. The weaker relationship between the unweighted N Index and mean NO_2 concentrations for the 12 week period was probably due to the local modification caused by the presence of the small waste water unit, which provided a significant contribution to NO_2 and NH_3 concentrations. Excluding this site as atypical, gave an excellent response of Ellenberg Index across the rest of the transect to NO_2 concentrations (R^2= 0.96), with a similar response to N deposition (R^2 = 0.81) and a weaker response to NH_3 (R^2 = 0.68).

Assessment using the Acidophyte/Nitrophyte index gave a clear indication of the impact of N attributed to road emissions, on the balance of acidophyte and nitrophyte species along the transect. At concentrations greater than 8 µg m^{-3} NO_2, nitrophytic species dominated the flora.

4.6.3. Bowbeat Hill and Auchencorth Moss

The mean Ellenberg N Index was small at these sites, indicative of low available N. Although blanket bogs are known to be naturally nutrient poor, large inputs of atmospherically deposited N may be expected to increase fertility and influence species composition. Atmospheric N deposition is small at Auchencorth Moss (10 kg N ha^{-1} y^{-1}) but at Bowbeat Hill, 584 m above sea level, N inputs are 2-3 times larger (25 kg N ha^{-1} y^{-1} (Crossley *et al.* 1992). Nevertheless the mean Ellenberg N Index was similarly small at both sites. Only in the comparison of cover weighted Ellenberg N index was a difference seen, but given the difference between sampling locations at Bowbeat Hill, this was not significant overall.

Changing N status at a site should drive change in mean Ellenberg N scores. However, if sampled vegetation contains only stress-tolerant, low N value species, changes may be modest or undetectable even if nutrient availability increases. At Bowbeat Hill blanket bog, the species sampled consisted almost entirely of stress-tolerant, slow-growing perennials with N values of 1 to 3. N deposition may have increased but these species appear to have responded by all increasing slightly in biomass resulting in unchanged unweighted and cover weighted mean Ellenberg scores. The presence of propagules of more responsive species may have resulted in more dramatic shifts in Ellenberg score both as a result of increased cover of new dominants and reductions in competitively inferior species.

Unpublished results (C. Pitcairn pers. comm.) suggest that upland mosses respond more closely to the concentrations of ammonium and nitrate in rainfall than to deposited N. Tissue N content of selected mosses increased in response to the experimental addition of N at concentrations that occur only in peak events. Exposure to concentrations commonly experienced on mountain-tops, resulted in very small increases in tissue N. Whether this response in tissue N can be related to changes in species composition requires further investigation.

Land management in some areas of Bowbeat Hill has resulted in loss of dwarf shrubs and increased cover of grass species (already present in small amounts). However as both dwarf shrubs and the grass species present are stress-tolerant and of low N value the mean Ellenberg N Index remained unchanged. However, application of the Acidophyte-Nitrophyte Index showed that acidophyte presence was considerably less on the grass dominated areas and on a cover basis nitrophyte cover almost equalled that of acidophytes. If growth conditions were to change in the future at Bowbeat Hill, (e.g. frequent frosts or summer droughts) resulting in a

decline in acidophytic dwarf shrubs, a considerable cover of potentially nitrophytic species already exists ready to exploit the decline.

While Ellenberg N Index gave no indication of the different atmospheric N loads received by the blanket bogs at Auchencorth Moss and Bowbeat Hill, the acidophyte/nitrophyte method, by concentrating on key N responsive species, may be more sensitive to changes in the N status of a site and able to provide an early warning of unwanted species composition shifts**.**

4.6.4. Method attributes and application

While the critical loads approach to conservation can indicate potential problems in a given area, biomonitoring methods can be used at the finer scale to validate critical loads exceedance at specific sites of conservation interest.

The Ellenberg N index is a robust indicator of enhanced N deposition as shown by the good relationship between the Ellenberg Index and N deposition for all the intensive sites. It has been used extensively in Europe to indicate vegetation change due to increased atmospheric N deposition (e.g. Ellenberg 1988; Tyler 1987; van Dobben 1993). At conservation sites, evaluation of the flora must be of major importance. A full species list can be easily used to determine the Ellenberg N Index of sites providing an important standard for comparison between sites, and within sites on a spatial and temporal scale.

Obviously the rigour and good practice of the surveyor are vital aspects in obtaining good results and agency staff should be trained in botanical identification. However, while a full botanical survey is necessary to obtain an accurate Ellenberg Index, a simpler index based on the acidophyte/nitrophyte balance may require less training and potentially be more sensitive to nitrogen-induced changes for a given habitat. Further development of key acidophyte and nitrophyte species for habitat types should facilitate application by conservation officers.

4.7. Conclusions

- The Ellenberg Index correlated well with atmospheric N deposition at the 4 intensive sites, confirming the strength of the method in indicating enhanced N deposition, and providing an important standard for comparison between sites, and within sites on a spatial and temporal scale.

- Ellenberg N Index provided a useful assessment of the N status of a site, particularly along known gradients in N deposition The determination of the Ellenberg N index along a gradient of ammonia concentration and N deposition at Piddles Wood, and a gradient of NO_2 concentrations and N deposition at Happendon Wood showed changes in vegetation composition which could be attributed to N deposition.

- Ellenberg N Index appears to be a weaker predictor of the relative N status of sites dominated by wet deposited N. At, Auchencorth Moss and Bowbeat Hill, blanket bog sites of naturally low N status, but with different atmospheric N inputs, mean Ellenberg N Index did not indicate any N driven change in species composition. At such sites, the presence of only stress-tolerant, low N value species and the absence of propagules of high NI plants may restrict changes in the mean Ellenberg NI in response to increased atmospheric N deposition.

- The determination of Ellenberg N values provides evidence of floristic change although the values cannot discriminate between different drivers of the detected floristic shift. The accuracy of the method is affected by other drivers of change, such as canopy type and cover, soil type and litter levels.

- The first attempt to apply an index based on the selection of acidophyte and nitrophyte species (vascular + bryophytes), as has been done for lichens, provided useful information on the eutrophication status of the sites. For example, at Piddles Wood the flora was shown to be dominated by nitrophyte species at NH_3 concentrations greater than 3 $\mu g\ m^{-3}$.

- The acidophyte/nitrophyte approach for higher plants, bryophytes and lichens has the potential to provide a more sensitive measure of N deposition induced changes, which is more relevant for target habitats. The next stage should be the development of a list of key species of acidophytes and nitrophytes for major habitats. Starting with woodlands, where considerable information is already available, further sites could be added to the database, followed by evaluation using a wide range of sites in the UK to develop the robustness and scope of this approach.

- The application of the both the Ellenberg N Index and acidophyte/nitrophyte method by agency staff requires training in botanical identification. However, while a full botanical survey is necessary to obtain an accurate Ellenberg Index, the simpler method based on the acidophyte/nitrophyte balance may require less training. Development of key acidophyte and nitrophyte species for habitat types should facilitate application by conservation officers.

5. Evaluating *Deschampsia flexuosa* as a standardised grass N bioindicator

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5.1. Introduction

The recommendations from the Phase 1 field study were to develop the wider application of standardised grasses as N biomonitors (Sutton *et al.* 2004a). Standardised grass plants can be used to evaluate the impact of N on a range of habitats. They are independent of local climatic and soil conditions and provide information on the scale of N impact at a specific site within a relatively short time period (0-3 months). The majority of N-based biomonitoring projects to date have used fast growing grasses biomonitors, such as *Lolium perenne, Lolium multiflorium* (Sommer and Jenson 1991; Sutton *et al.* 2004a). The use of such species has been successfully applied in short-term studies investigating strong localised agricultural point sources.

However, such fast growing species are not suitable for longer term studies in more exposed upland areas subjected to diffuse N input and a high episodicity of wet N deposition. Therefore, there is a requirement to identify and test slower growing grass species for use as potential standardised grass bioindicators in long-term N studies. The objective of the current study was to test the suitability of the grass species, *Deschampsia flexousa* as a standardised grass bioindicator species for a range of habitats and atmospheric N pollutant inputs.

5.2. Methods

5.2.1. Plant material

Deschampsia flexuosa (L.) Trin. (common name: Wavy hair-grass) is a slow growing, evergreen, tufted or mat-forming polycarpic perennial grass. It is the most successful calcifuge grass species in the UK and is found in a wide range of unproductive habitats. It is very abundant in moorland, acidic heaths, open-woodland and plantations, but normally in drier areas of these habitats. Within moorland habitats it is often associated with *E. vaginatum* and *C. vulgaris* and is tolerant of low soil pH, and has a low nutrient mineral requirement. It can also occur in acidic soil habitats such as neglected hill pasture, cliffs and spoil heaps, but is normally absent from farmland. The shoots/tillers are hair like in appearance, erect and up to 200 mm in length. It can be grazed by sheep and rabbits, but when it is in association with other species such as *C. vulgaris*, the ericoid will be grazed preferentially. *D. flexuosa* flowers between June and July and is found across a wide altitudinal range; from low altitudes up to 1000 m in mountainous areas of Scotland, however, it is rarer in southern Britain and mainly absent in the south-east.

5.2.2. Propogation

Tillers from mature plant stock of *Deschampsia flexuosa* were propagated at CEH during the summer/autumn of 2003. The *D. flexuosa* seed had been originally supplied by Herbiseed, The Nurseries, Billingbear Park, Wokingham, Berkshire, RG 40 5RY. The rooted young plants were transferred to 1.1 litre square black pots containing a peat: loam: grit compost (ratio 4:1:1) with no added fertiliser once they had rooted and had begun to develop. All the

individual pots had a wicking system added providing irrigation on demand. All plants were over-wintered in an unheated glasshouse, and then hardened off in early spring 2004, when they were transferred outdoors prior to use at the 5 intensive sites.

5.2.3. Irrigation system

As all the intensive sites would only be visited irregularly (7-10 days between visits), an irrigation system had to be designed which would provide the plants with sufficient water between visits. The wicking system with a reservoir of water was based on the EuroBioNet system (see EuroBionet instruction manual for details; www.eurobionet.com). Two pieces of glass fibre cord (250 mm in length) were placed into each individual pot, with both wicks running vertically from just below the soil surface down to the water tray reservoir. The wicks were in constant contact with the rainwater reservoir and therefore, kept the soil moist even during dry warm periods (Figure 5.2b). All the monitoring positions at each of the extensive study sites (i.e. Piddles Wood, Happendon Wood, Auchencorth Moss and Bowbeat Hill) comprised of a single set of monitoring plants (consisting of six *D. flexuosa* six plants per tray). The six plants were placed into a polystyrene moulding, which supported the plants above the 600 x 400 x 145 mm HDPE water reservoir tray (volume 28 litres) thus allowing each plants wick to absorb the rainwater that normally was used to fill the reservoir (Figure 5.1). At Whim Moss, smaller two plant trays (400 x 300 x 145 mm, HDPE reservoir tray (volume 13 litres) with identical polystyrene moulding and wicking system were used in both the dry and wet studies because of three replicated plots per wet treatment (Figure 5.2). All trays were protected from wildlife by 13 mm^2 Netlon garden mesh supported by 60 cm wooden posts (Figure 5.1 and 5.2a).

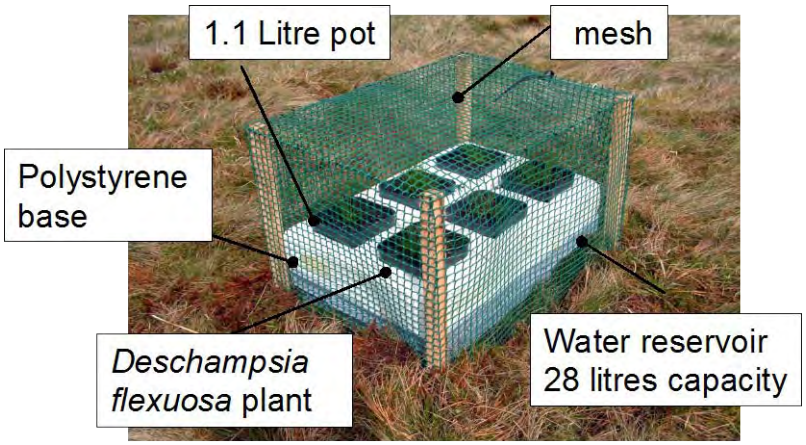
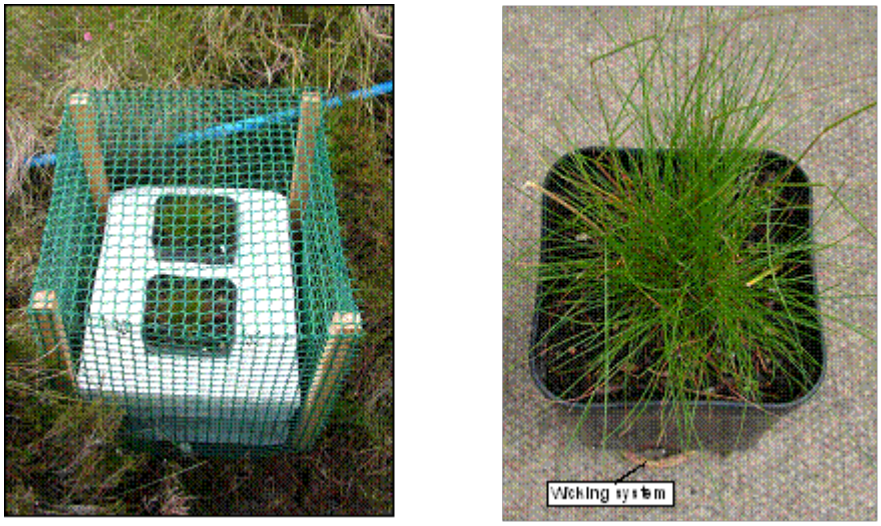


Figure 5.1. Photograph of the six-plant system used at Piddles Wood, Happendon Wood, Auchencorth Moss and Bowbeat Hill.

5.2.4. Pre-treatment harvest

On the 25 April 2004, two bulk pre-treatment samples were taken from 10 random plants for total tissue N concentration and soluble NH_4-N concentration thus providing a baseline for total tissue N content prior to pollutant exposure.



a b

Figure 5.2. a) Close-up photograph of the 2 standardised plant system used at Whim Moss and b) a photograph of a typical *D. flexuosa* plant after 12 weeks of exposure.

5.2.5. Experimental procedures

The standardised grass biomonitors studies at each of the 5 intensive sites ran for a 12-week period. However, due to the wide geographical distribution of the sites, all sites could not be set-up in the same week, but were established over a 3-4 week period in spring 2004.

Piddles Wood

The grass biomonitoring system was established at Piddles Wood on 26 April 2004 with a single tray of *Deschampsia flexuosa* (as described in Section 5.2.3) placed at each of the four distances (5, 20, 40 and 100 m) along a 250 m transect running in a NE direction away from the poultry unit. One tray (6 x *D. flexuosa* plants) was set-up at each of the four distances (Figure 5.3). The additional site at 250 m distance from the poultry house was established 6 weeks into the study, as it was suspected that there could still be an influence of the poultry unit on the lichen flora at the 100 m distance. However, additional grass biomonitors were not placed at this distance, as it would have been impossible to interpret the growth and nutrient data with the different exposure periods. A local consultant was used to maintain the grass biomonitors. The biomonitor plants were collected and harvested on 21 July 2004.

Happendon Wood

This site was set-up on 19 April 2004, with trays of *D. flexuosa* (as described in Section 5,2.3.) were placed at 5 distances (10, 20, 38, 200 and 250 m along a 250 m transect running in a NE direction away from the M74 motorway at Happendon Wood on 19 April 2004 and run for 12 weeks until 3 July 2004. These sites are listed as site 1, 2, 3, 4 and 5 in Figure 5.4. An additional site at 150 m (Site 6) distance from the M74 was established 6 weeks into the study to determine the influence of the B7078 on the lichen diversity and N deposition effects on pleurocarpous mosses. No *D. flexuosa* plants were positioned at this site. Staff from CEH Edinburgh maintained the plants and visited the site every 7-10 days.

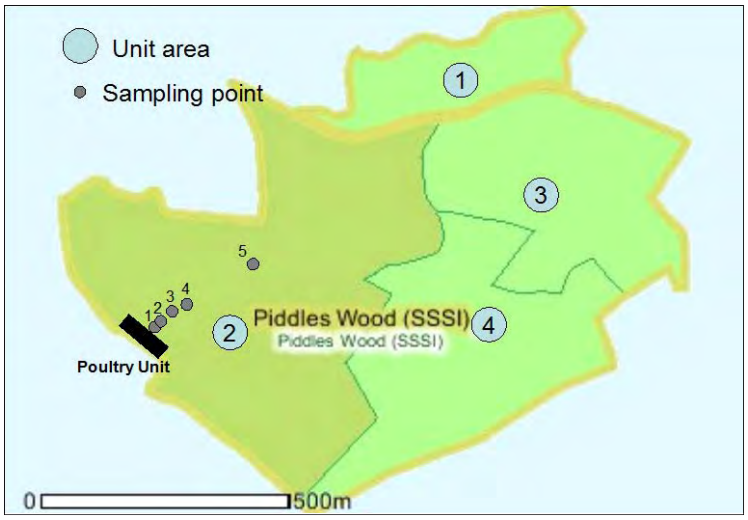


Figure 5.3. Schematic diagram showing the SSSI's four unit areas and the locations of the standardised grass plants 1-4 (5, 20, 40, 100 m from poultry unit respectively) at Piddles Wood. Site 5 (250 m from the point source) was established after 6 weeks of the study as an additional ambient background site for use in the lichen diversity and pleurocarpous moss studies.

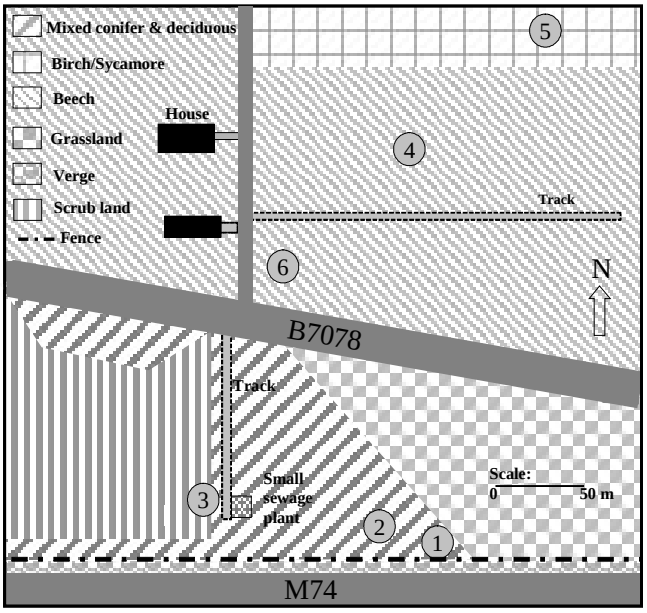


Figure 5.4. Schematic diagram showing the distribution of the 5 standardised plant sites at Happendon Wood. Each set of plants were placed at sites 1, 2, 3, 4 and 5 (10, 20, 38, 200 and 250 m from the motorway). Site 6 (150 m) was added after 6 weeks to determine the influence of the B7078 (20 m away) on the lichen diversity and N deposition in pleurocarpous mosses.

Auchencorth Moss and Bowbeat Hill

A single 6-plant tray of standardised *D. flexuosa* plants was placed out in the fenced CEH Experimental facility at Auchencorth Moss on 29 April 2004. The plants were exposed until 22 July 2004.

A single tray of standardised *D. flexuosa* plants was placed out at the experimental upland site at Bowbeat Hill on 5 May 2004. The plants were exposed for 12 weeks until 28 July 2004. CEH Edinburgh staff maintained the plants visited this site weekly.

Whim Moss

Standardised *D. flexuosa* plants were exposed in both wet and dry N deposition treatments at the Whim Moss N field manipulation facility using the two plants per tray system (Figure 5.2). The site was established as a long-term field study into the effects of different N forms to *Calluna vulgaris/Eriophorum vaginatum* vegetation (NVC; M19a) and is currently funded as part of the Defra Acidification and N umbrella programme (www. bangor.ac.uk/terrestrial-umbrella). A full description of the site and the NH_3 fumigation/wet N deposition facility are described by Leith *et al.* (2004) and Sheppard *et al.* (2004).

Dry NH_3 treatments

Using the NH_3 field release system, standardised *D. flexuosa* plants were set-up at 5 distances (6 m, 16 m, 32 m, 60 m, 60 m 12 m east) along the NH_3 gradient and at background (60 m west, behind the NH_3 release point) ambient on 3 May 2004 (Figure 5.5). A two-plant tray was placed at each of these 6 sampling points. NH_3 concentrations are monitored using NH_3 passive samplers at distances of 2, 4, 6, 8, 12, 16, 32, 60 m along the 60 m NH_3 gradient. As it is not possible to increase the length of the transect additional lower NH_3 concentration sites have been added at 12 and 15 m east and west of the 60 m distance. The grass biomonitors were set-up at distances, with existing NH_3 concentration monitoring. Section 3.6 details the NH_3 monitoring at Whim Moss. The automatic NH_3 field release system is programmed to release NH_3 gas when the wind direction is in the sector 180^0-215^0 and the wind speed is > 2.5 m s^{-1}. Therefore, the duration of the NH_3 release in any given month is totally dependent on the prevailing weather conditions during that month.

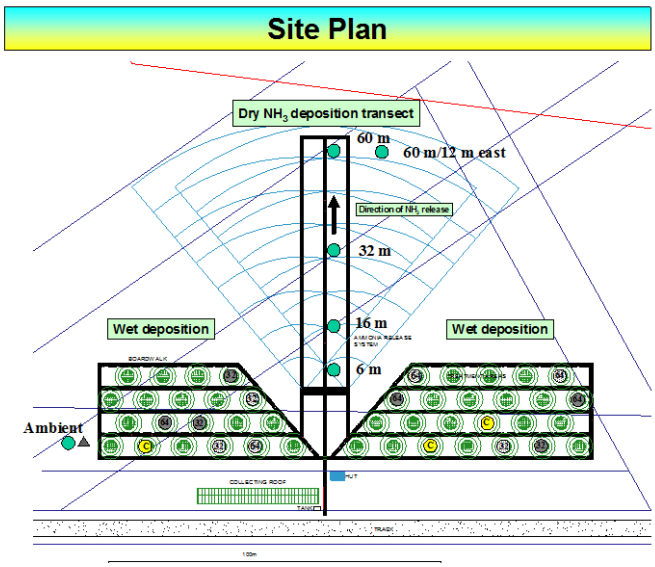


Figure 5.5. The distribution of the standardised grass biomonitors (*D. flexuosa*) along the NH_3 and the experimental layout of the wet N deposition plots at Whim Moss.

Whim Moss: Wet N treatments

The wet N deposition field release system at Whim Moss was used (Sheppard *et al.* 2004) to expose a total of 15 two–plant trays of standardised grass biomonitor *D. flexuosa*, to a range of three wet N deposition (control (10 kg N ha^{-1} y^{-1}), 32 kg N ha^{-1} y^{-1} and 64 kg N ha^{-1} y^{-1}) and 5 treatments applied as either NH_4Cl or $NaNO_3^-$ (Table 5.1). The standardised grass biomonitors study (2 replicate plants per N treatment plot and 3 replicate N treatment plots) was set up on 18 May 2004 and ran for 12 weeks until 11 August 2004. The *D. flexuosa* plants were placed at the edge of the existing experimental plots, but received the same N dose as plants within the treatment plot (Figure 5.6 and Figure 5.7). As with the dry NH_3 treatments, the frequency of application of wet N treatments is totally dependent on the

prevailing meteorological conditions. Treatments were applied only when sufficient precipitation had been collected and the wind speed was < 5.0 m s^{-1}. The frequency of application during the exposure period of the *D. flexuosa* study at Whim Moss is shown in Figure 5.8. The Bowbeat Hill precipitation data collected using a tipping bucket rain gauge is shown in Figure 5.9.

Table 5.1. Wet N deposition treatments used in the standardised *D. flexuosa* study at Whim Moss.

Treatment deposition (kg N ha^{-1} y^{-1})	N deposition during exposure period (kg N ha^{-1} y^{-1})	N treatments applied as:	Number of replicate treatment plots
Control (10)	2.4	Rainfall	3
32	4.5	$NaNO_3^-$	3
32	4.5	$NaNO_3^-$	3
64	9.5	NH_4Cl	3
64	9.5	NH_4Cl	3

Figure 5.6. Photograph of wet treatment plot *and D. flexuosa* standardised plants at Whim Moss.

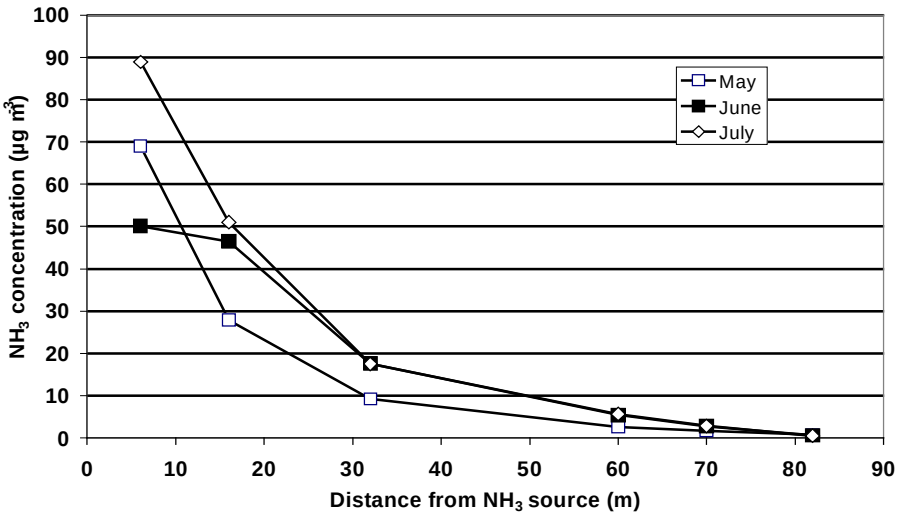


Figure 5.7. The mean monthly atmospheric NH_3 concentration data for May, June and July 2004 at Whim Moss N field manipulation site.

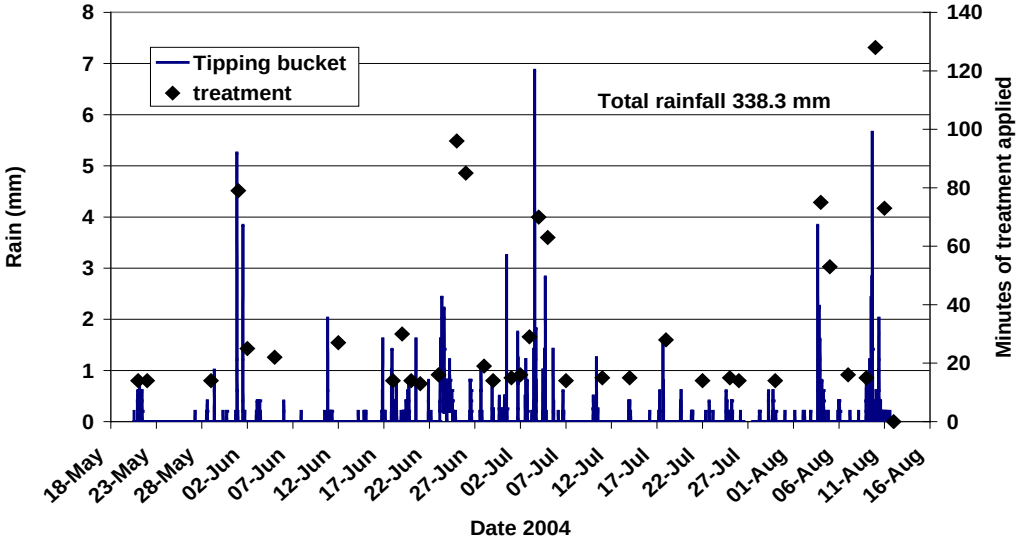


Figure 5.8. Whim Moss 15 minute average rainfall data and minutes of applied wet N deposition treatment, 20 May to 11 August 2004.

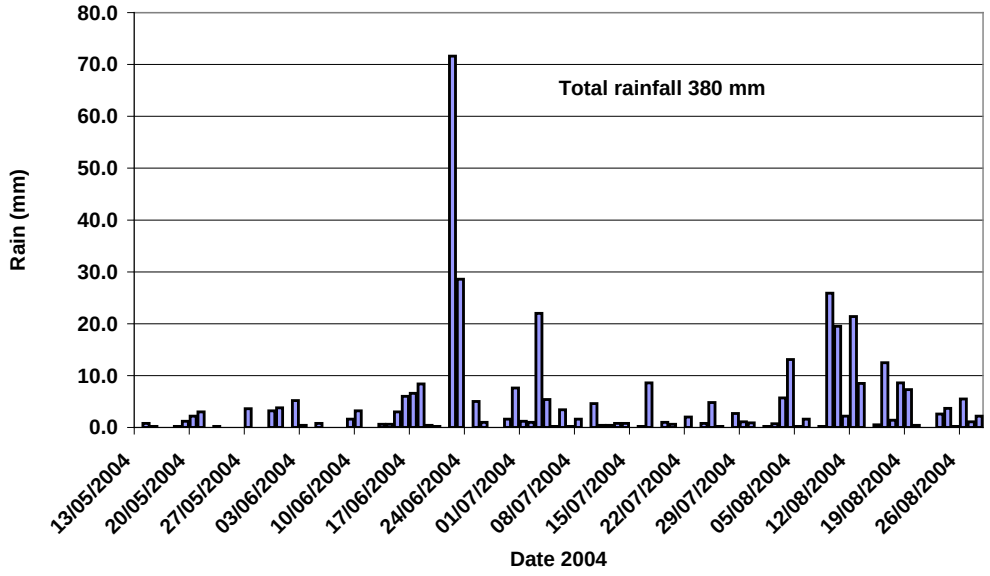


Figure 5.9. The rainfall data (20 minute average) at Bowbeat Hill for the exposure period 13 May to 26 August 2004.

5.3. Results

Piddles Wood

5.3.1. Biomass determination

The standardised *Deschampsia flexuosa* plants at Piddles Wood were found to be strong and healthy at all points along the 100 m transect after 12 weeks of exposure. A strong log–linear relationship was found between biomass accumulation and distance at the 5, 20 and 40 m from the poultry house (Figure 5.10). Biomass accumulation increased in the vicinity (5 m) of the poultry unit by approximately 30% compared with those plants at 40 m from the poultry unit. Biomass values for 100 m from the poultry unit was very different due to the very different shading levels compared to the other sites, and has therefore been plotted separately. The canopy structure at the 100 m site was very open when the standardised plants were set-up in May 2004 and therefore probably encouraged increased growth compared to the more shaded sites (Figure 3.4).

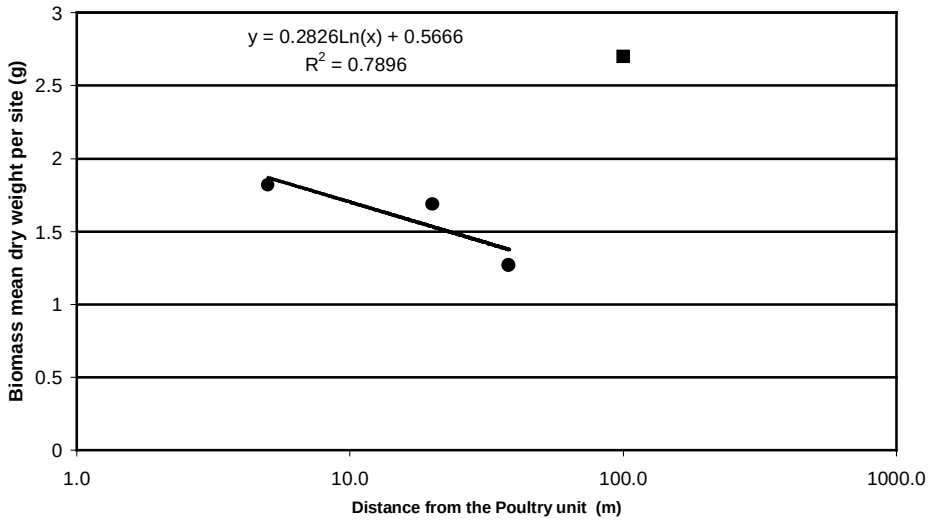


Figure 5.10. The relationship between biomass accumulation of standardised *D. flexuosa* biomass and log distance from the poultry unit at Piddles Wood after 12 weeks of exposure**.**

Consistent with the increase in NH_3 concentration near the poultry unit, the biomass of the plants increased with increasing NH_3 concentration (Figure 5.11), apart from the 100 m point, (see Section 5.3.1).

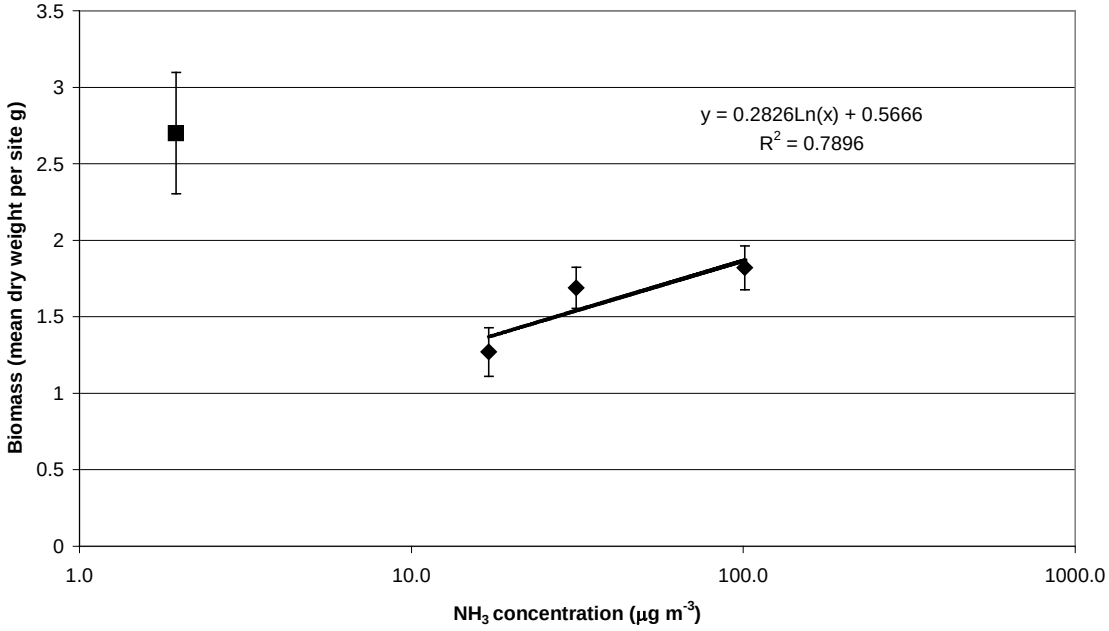


Figure 5.11. *Deschampsia flexuosa* biomass increase with the log NH_3 concentration away from the poultry unit at Piddles Wood after 12 weeks of exposure**.**

The matching relationship for N deposition and biomass is shown in Figure 5.12), with the N deposition values as estimated in Section 2. Plant biomass accumulation increased with increasing total N deposition rates.

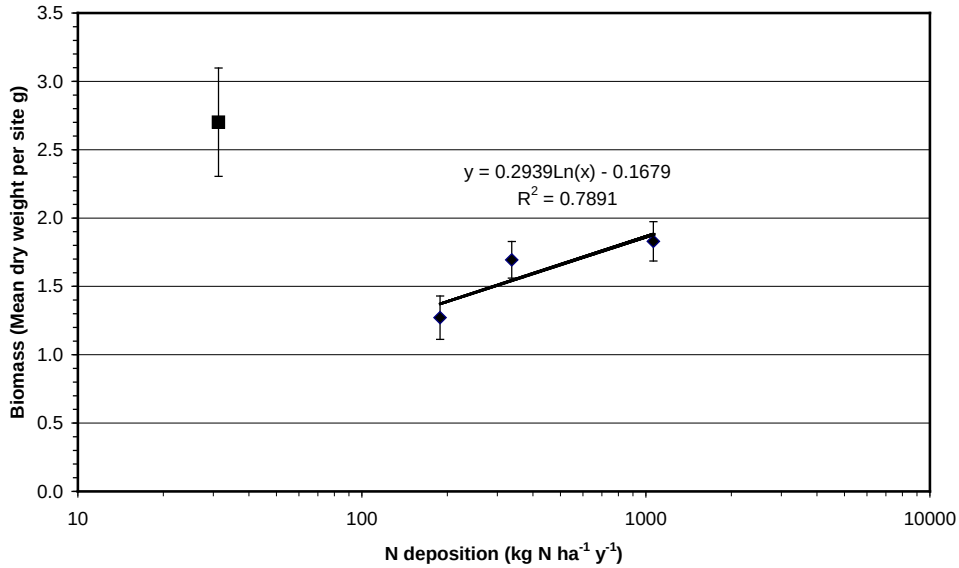


Figure 5.12. The relationship of *D. flexuosa* biomass expressed as NH_3 concentration after a 12 week exposure period at Piddles wood, Dorset.

5.3.2. Tissue N content (% dry weight) and NH_4-N concentration

There is a good log–linear relationship with tissue N content and soluble NH_4-N concentration with distance from the poultry farm. The concentrations of tissue N content dramatically reduced with distance by 50% from 2.86% N at 5 m from the poultry unit to 1.43 % N at 100 m (Figure 5.13).

A similar response was found in the soluble NH_4-N with the concentration decreasing from 10.2 to 3.1 μg g^{-1} FW (Figure 5.13).

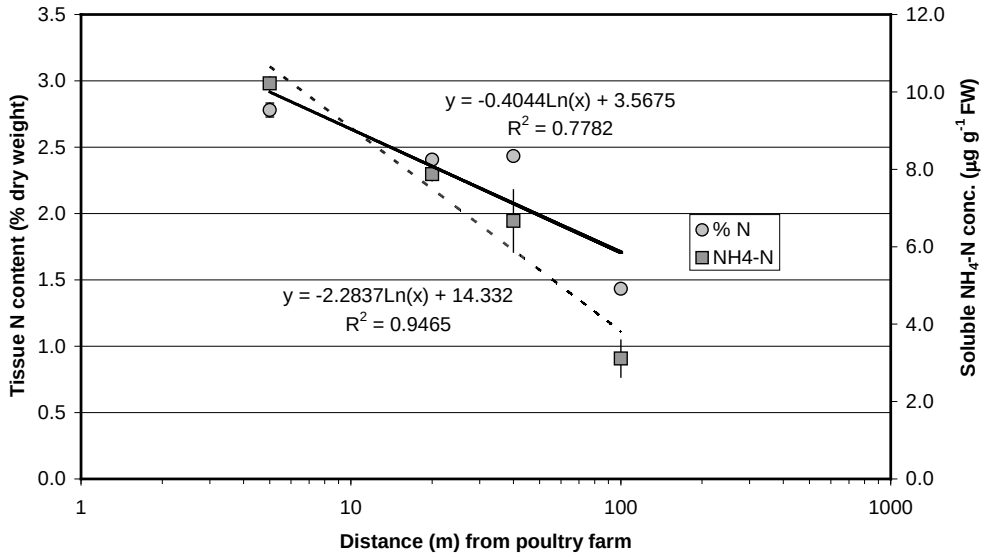


Figure 5.13. The tissue N content and the soluble NH_4-N concentration (after 12 weeks of exposure) with distance (m) from the poultry unit at Piddles Wood, Dorset. The dotted line represents the relationship with soluble NH_4-N concentration and the solid line, tissue N content.

There were very strong relationships between both tissue N content and soluble NH_4-N concentration and NH_3 concentration and total N deposition (Figures 5.14 and 5.15). In contrast to the results for biomass (Figures 5.10-5.12), the tissue N and soluble NH_4-N concentration results (Figure 5.13-5.15) were consistent across the whole transect, including the site 100 m distant from the poultry house. Overall the highest correlation (R^2 = 0.999) was for the relationship between foliar ammonium concentrations and NH_3 concentration measured during the exposure period.

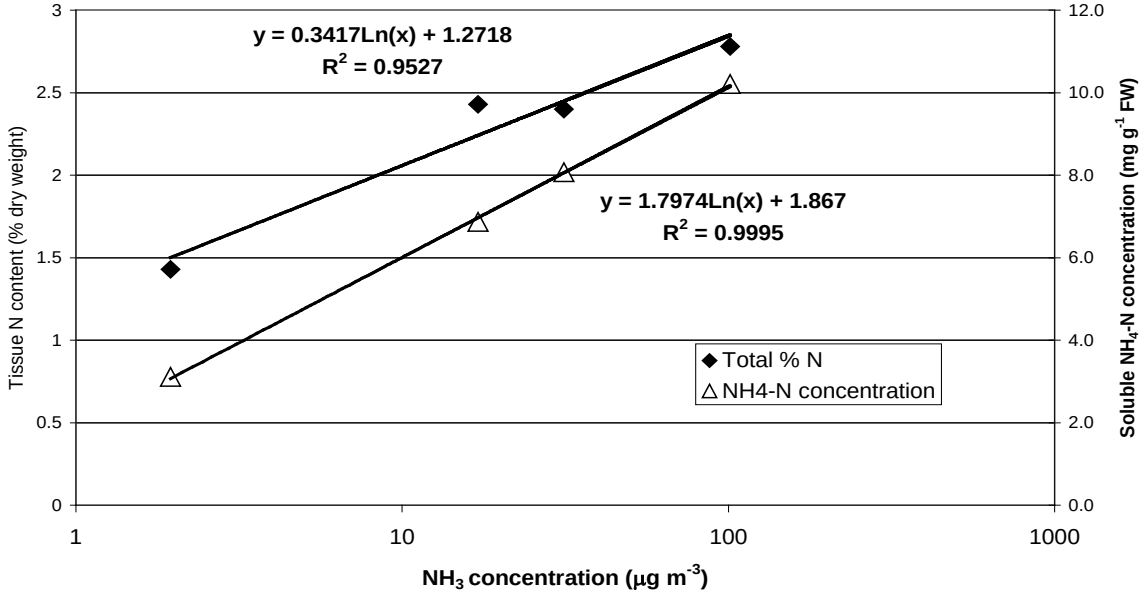


Figure 5.14. The relationship between tissue N content and the soluble NH_4-N concentration in *D. flexuosa* (after 12 weeks of exposure) and atmospheric NH_3 concentration at Piddles Wood, Dorset.

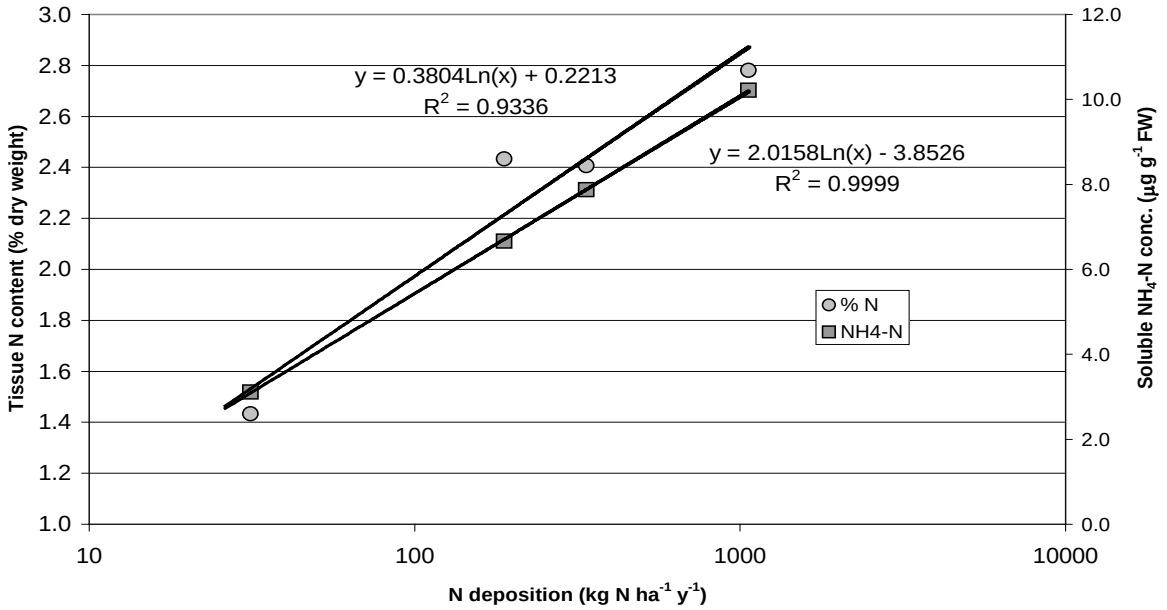


Figure 5.15. The relationship between tissue N content, the soluble NH_4-N concentration and N deposition in *D. flexuosa* (after 12 weeks of exposure) at Piddles Wood, Dorset.

The Piddles Wood data for standardised grass *Deschampsia flexuosa* are summarised in Table 5.1. When the tissue N content data and the biomass data are expressed as mg N per pot, results at the 100 m point are again very different from the log–linear relationship found for the 5-40 m sites for both NH_3 concentration and N deposition (Figures 5.16 and 5.17). The mean tissue N content at 100 m was lower (1.43% N) compared to the 40 m value (2.43% N), but the biomass at 100 m is larger than all the other points. The large difference in biomass is thought to be due to the different levels of shading at 100 m compared to the other 3 sites, which had comparable levels of shading. A summary of the Piddles Wood standardised grass study data are shown in Table 5.2.

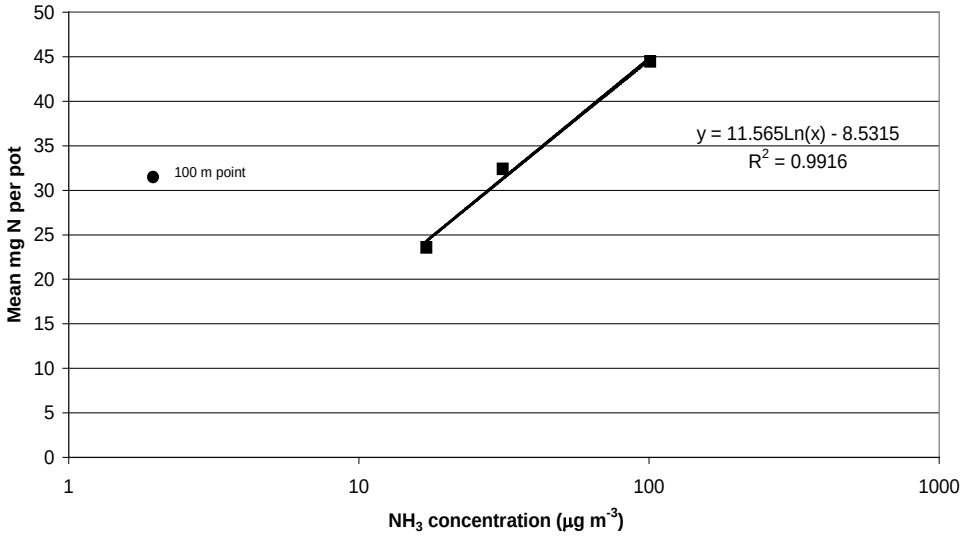


Figure 5.16. The relationship of *D. flexuosa* biomass expressed as mg N per pot with NH_3 concentration after a 12 weeks exposure period at Piddles Wood, Dorset.

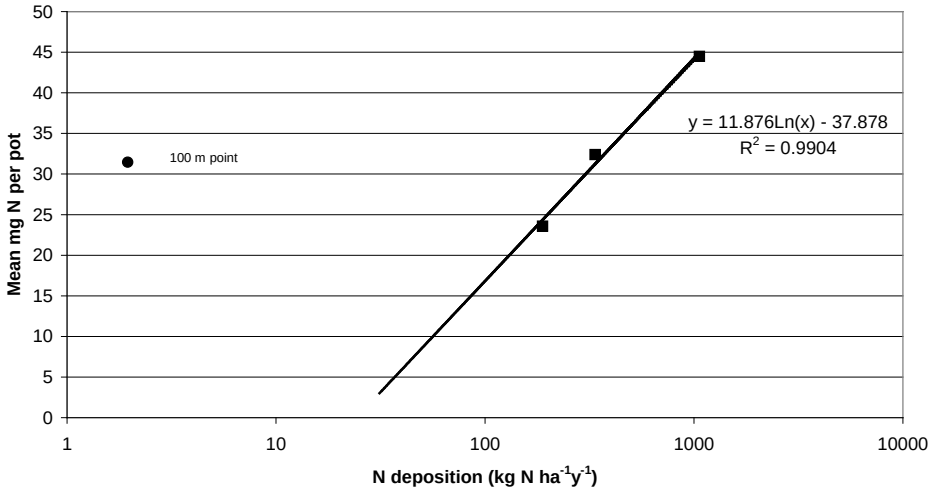


Figure 5.17. The relationship of *D. flexuosa* biomass expressed as mg N per pot with N deposition after a 12 weeks exposure period at Piddles Wood, Dorset.

Table 5.2. Data for Piddles Wood standardised grass transplant study with *Deschampsia flexuosa* along a NH_3 gradient from a poultry unit.

Site No.	Distance	Mean Biomass (g)	% N content	Soluble NH_4-N conc.	Total N inventory
1	5	1.82	2.78	10.2	45
2	20	1.69	2.40	8.1	33
3	40	1.27	2.43	6.9	24
4	100	2.70	1.40	3.1	31
5	250				
R^2 with log distance		0.78	0.78	0.95	0.99
R^2 with log NH_3 concentration		0.79	0.95	0.99	0.99
R^2 with log N deposition.		0.79	0.93	0.99	0.99

Happendon Wood

Standardised *Deschampsia flexuosa* plants were placed out at five locations along the 250 m transect (Figure 5.4). The level of shading varied along the transect with the sites at distances 10, 20 and 38 m being shaded in the conifer dominated stand, whereas at 200 m from the motorway the mature oak and beech woodland was more open. The site at 250 m was in more juvenile woodland, which was less exposed than the 200 m site.

5.3.3. Biomass determination

The above-ground biomass *increased* linearly with log distance from the M74 motorway. There was a large difference in the biomass at the two positions in the beech/oak woodland (sites 4 and 5) compared to the three in the mixed conifer sites (Figure 5.18). This is in contrast to the results at Piddles wood, where biomass decreased with distance from the N source. This may either be due to other negative effects of emissions from the M74 motorway (e.g. salt, particles, NO_2 concentrations) or related to the different levels of shading along the transect. Hence the different habitats may be as influential in the measured biomass increases as N pollution. The implications of a non-uniform habitat type along the pollution gradient is therefore of importance when considering the impacts of N pollution from a point source.

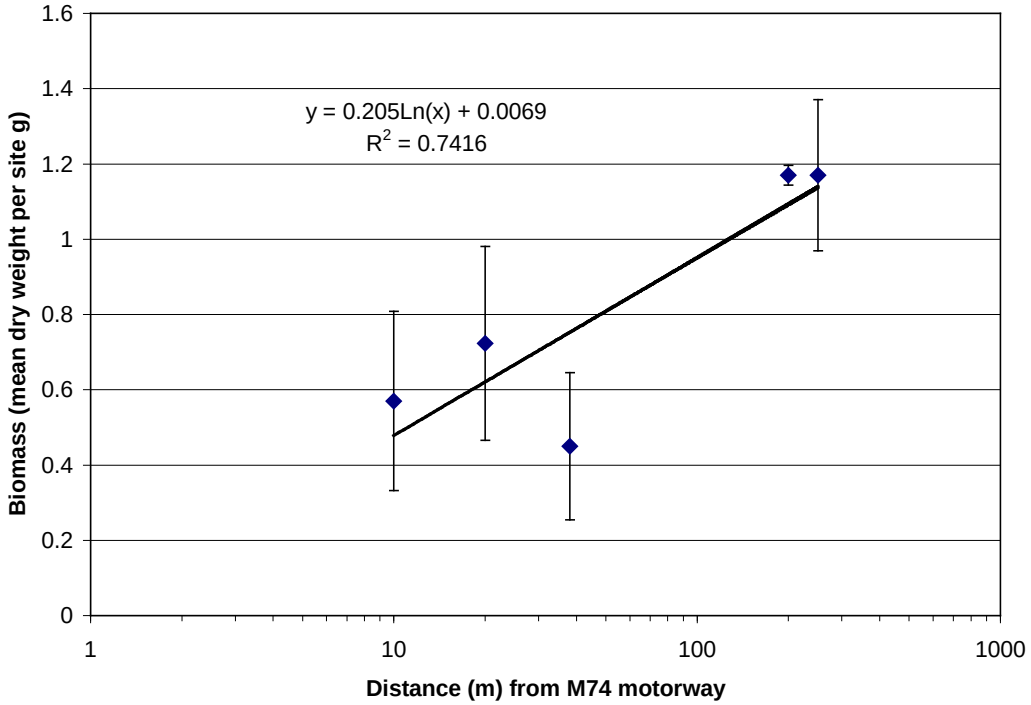


Figure 5.18. *Deschampsia flexuosa* biomass accumulation after 3 months of exposure along a 250 m transect from the M74 motorway at Happendon Wood.

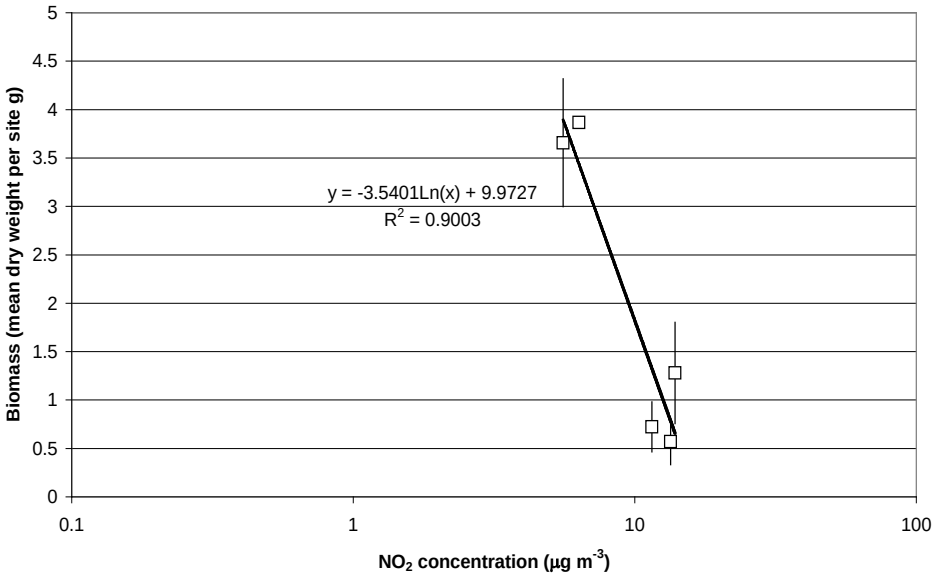


Figure 5.19. Impact of NO_2 concentrations on *Deschampsia flexuosa* biomass accumulation after 3 months of exposure along a 250 m transect from the M74 motorway at Happendon Wood.

The above ground biomass of the standardised *D. flexuosa* decreased significantly at the set distances along the 250 m transect with increasing NO_2 concentration (Figure 5.19). Although, there was a reasonable relationship with biomass and NH_3 concentration (R^2 = 0.695), the strong relationship with NO_2 concentration (R^2= 0.900) suggests that NO_2 concentration rather than NH_3 concentration is influencing above ground growth in *Deschampsia flexuosa.*

Above ground biomass in the standardised *D. flexuosa* plants was shown to decrease with increasing log N deposition along the transect (Figure 5.20). It is suspected that it is not only N deposition that is the casual factor in this decrease in biomass. The impacts of the other

vehicular pollutant emissions associated with the motorway and the variation in habitat (type and density) may also contribute towards this decrease in biomass accumulation.

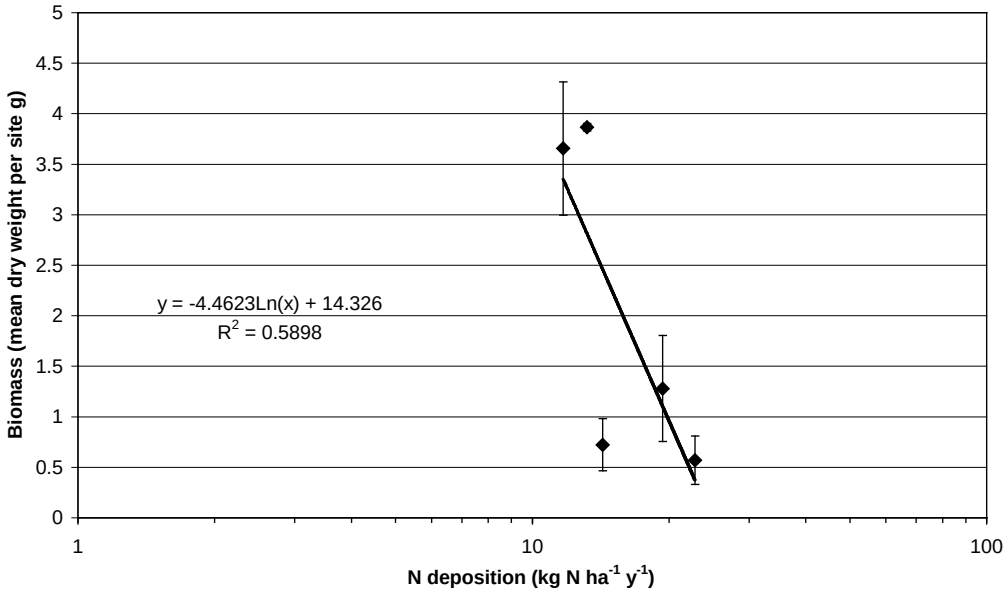


Figure 5.20. A decrease in the above ground biomass of *D. flexuosa* with increasing N deposition at Happendon wood, south Lanarkshire.

Interestingly, calculation of the above ground N inventory shows a decrease in total N per mg of plant biomass with increasing log N deposition, however, this relationship is not strong (Figure 5.21).

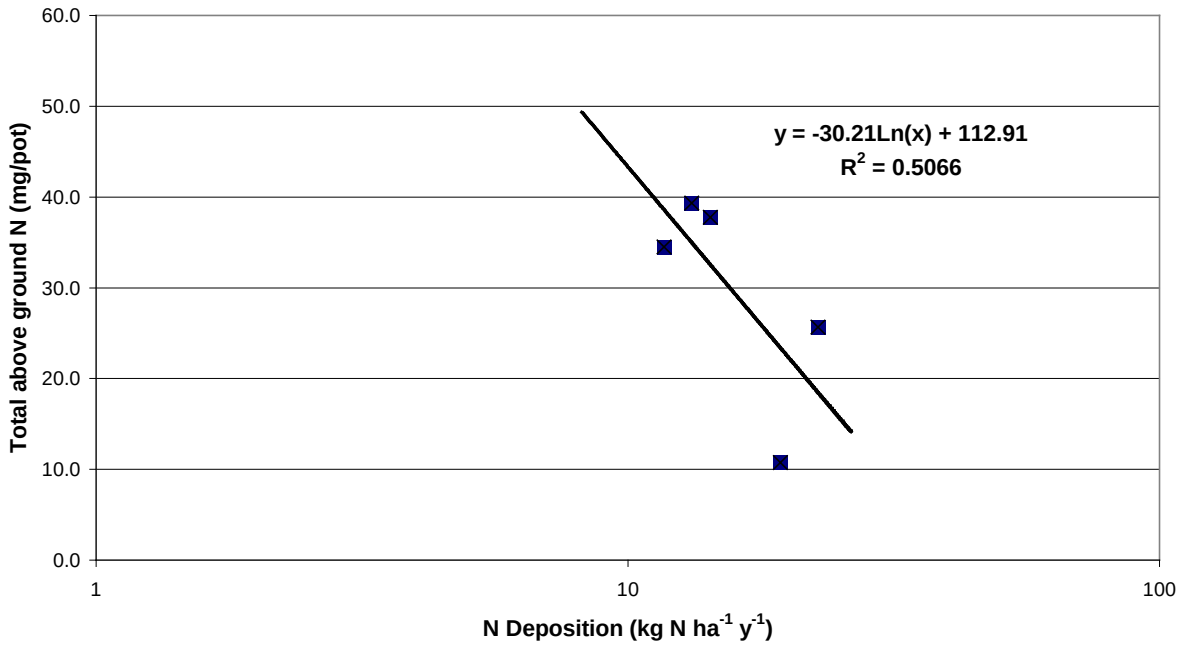


Figure 5.21. The relationship of *D. flexuosa* biomass expressed as mg per pot with N deposition after a 12 week exposure period at Happendon Wood, south Lanarkshire.

5.3.4. Tissue N content (% dry weight) and soluble NH_4-N concentration

There were strong relationships between distance from the motorway, NO_2 concentration and N deposition and tissue N content but not for NH_3 concentration (see Table 5.2). There was a strong relationship between NO_2 and tissue N content (Figure 5.22). There was no relationship between soluble NH_4-N concentration and NH_3 concentration, NO_2 concentration

and N deposition. All parameters for soluble NH_4-N concentration had R^2 of <0.200 (Table 5.3). This result would suggest that NH_4-N concentration is not a good chemical bioindicator of N impacts when NO_2 is the dominant form of N at a site. As site 3 was close to a pumping station the data for this site was not included the analysis but is shown in the Figure 22.

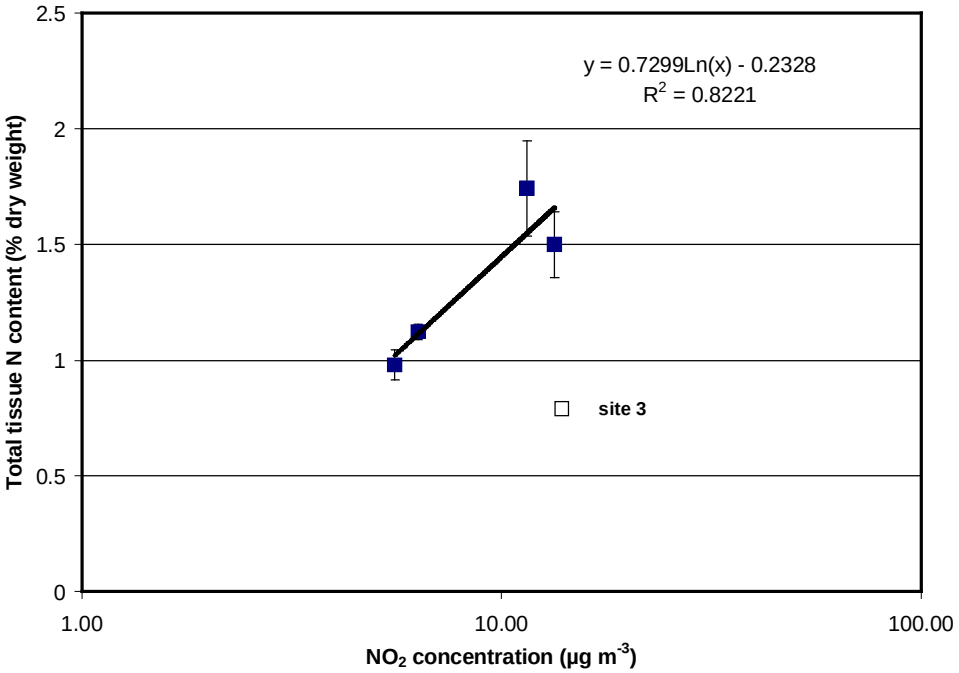


Figure 5.22. The relationship between NO_2 concentration and tissue N content in *Deschampsia flexuosa* standardised grass transplants along a gradient away from the M74 motorway at Happendon Wood, South Lanarkshire.

A summary of the *Deschampsia flexuosa* data for Happendon Wood is found in Table 5.2, however measurements of atmospheric NO_2 and NH_3 concentrations and total N deposition along the transect from the motorway are presented in full under Section 3.3.3 and summarised in Table 3.4.

Table 5.3. Summary data for Happendon Wood standardised grass study with *D. flexuosa*.

	Site No.	Distance	Biomass (g)	% N content	Soluble NH_4-N conc.	Total N inventory
	1	10	0.57	1.50	2.74	25
	2	20	0.72	1.74	1.88	37
	3	38	0.45	0.79	3.51	10
	4	200	1.17	1.12	3.45	39
	5	250	1.17	0.98	2.34	35
R^2 with log distance			0.742	0.787	0.142	0.368
R^2 with log NO_2 concentration			0.900	0.822	0.122	0.389
R^2 with log NH_3 concentration			0.695	0.230	0.031	0.500
R^2 with log N deposition			0.589	0.291	0.006	0.507

Auchencorth Moss and Bowbeat Hill

5.3.5. Biomass determination

Although, there was no significant difference between these two sites (Auchencorth Moss and Bowbeat Hill) for total biomass (P=0.097), the total above-ground biomass was greater at Auchencorth Moss than Bowbeat Hill. This was probably due to the difference in altitude between the sites, with Auchencorth Moss being less exposed and having slightly higher temperatures than Bowbeat Hill. Some of the tillers on the plants exposed at Bowbeat Hill had

a red colouration, a typical visible sign of increased concentrations of anthocyanin, indicating that the plants were under stress (although not directly analysed).

5.3.6. Tissue N content (% dry weight) and NH_4-N concentration

The mean tissue N content in the standardised plants was higher at Bowbeat Hill than Auchencorth Moss at 0.81% N and 0.65% N respectively, however, this was not significant (p=0.251). The difference in N would indicate greater uptake efficiency at Bowbeat Hill, although this difference could be attributed to a reduced growth rate at Bowbeat Hill.

5.3.7. N inventory at Bowbeat Hill and Auchencorth Moss

The above ground N inventory was very low at both sites at 4.3 and 4.4 mg per pot respectively for Auchencorth and Bowbeat Hill. This is approximately 10 times lower than was found at Piddles Wood (Table 5.1) indicating the influence of both atmospheric N pollution and habitat on N uptake. As a result of exposure and temperature, there would be a lower growth rate at both Auchencorth and Bowbeat Hill compared to Piddles Wood. The % N content at both these sites was also much lower compared to the 100 m site Piddles Wood, which was considered to be at ambient background concentration.

There was virtually no difference in the soluble NH_4-N concentration between Bowbeat Hill and Auchencorth Moss in the above-ground foliage (Table 5.4). Both were very low (2.2 and 2.3 $\mu g\ g^{-1}$ FW respectively). The low concentrations may reflect the diffuse N source and a possible slow uptake of N due to the exposure. This result indicates that for a site with a diffuse wet dominated N source, 3 months exposure is insufficient to detect differences between a site with annual N inputs of 25 kg N $ha^{-1}\ y^{-1}$ and 14 kg N $ha^{-1}\ y^{-1}$ when using grass biomonitors with the degree of replication applied here.

Table 5.4. Summary data for the two long-range N source sites at Auchencorth Moss and Bowbeat Hill.

Site Name.	N deposition	Biomass (g)	%N content	Sol NH_4-N conc.	Total N inventory
Auchencorth Moss	14	2.07	0.65	2.3	4.3
Bowbeat Hill	25	1.72	0.81	2.2	4.4
Probability of difference		p=0.690	p=0.251	p=0.87	p=0.976

(Single factor Analysis of variance: n=3)

Whim Moss

The use of the Whim Moss manipulation experimental facility presented a unique opportunity to expose standardised grass plants to different forms of wet N inputs under controlled N conditions and the same climatic conditions. The levels of wet and dry N deposition to the NH_3 transect and the wet N plots were dependent on the prevailing weather conditions during the 3 months exposure period.

5.3.8. NH_3 transect: Biomass determination

There was no effect on *D. flexuosa* above ground biomass with increasing NH_3 concentration, N deposition or distance along the 60 m transect after 3 months exposure (Table 5.5). However, there was a reasonable relationship (R^2=0.497) with distance and N inventory but this was not shown for N inventory and NH_3 concentration (R^2 =0.079).

Table 5.5. Summary table of the dry N deposition (kg N ha^{-1} y^{-1}) results of the standardised grass biomonitors study at Whim Moss, Scottish Borders.

Site No.	Distance from NH_3 source (m)	Biomass	% N content	Soluble NH_4-N conc.	Total N inventory
1	6	2.0	1.27	4.8	13
2	16	1.3	1.21	2.9	8
3	32	1.0	0.79	2.8	7
4	60	0.6	1.01	2.8	6
5	60 m +12m East	1.6	0.70	3.0	7
6	Ambient	0.7	0.91	2.0	9
R^2 with log distance		0.042	0.016	0.673	0.497
R^2 with log NH_3 concentration		0.001	0.451	0.563	0.079

5.3.9. NH_3 transect: Tissue N content and NH4-N concentration.

The standardised grass plants exposed along the NH_3 gradient showed an increase in tissue N content with increasing NH_3 concentration, although the linear relationship with tissue N content and log of NH_3 concentration was relatively weak (R^2 = 0.453; Figure 5.23). The estimated total N deposition for the 3 month exposure period was 0.6-58 kg N ha^{-1} y^{-1} respectively for the ambient and 6 m treatment positions along the NH_3 transect.

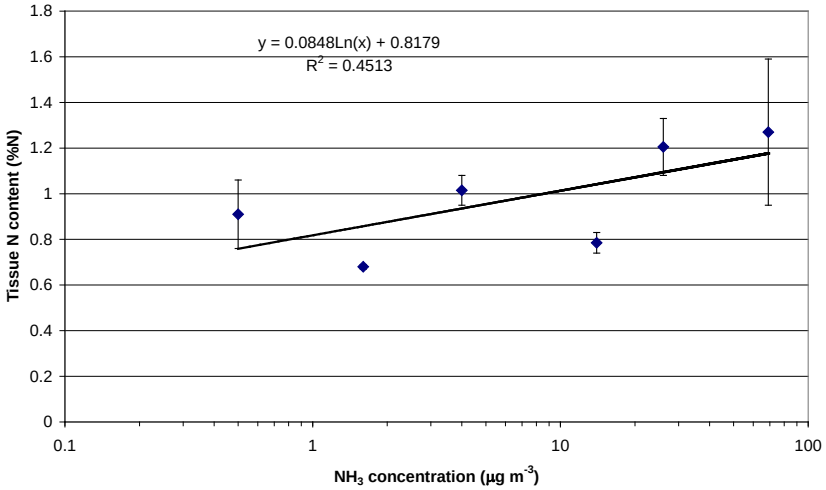


Figure 5.23. The mean tissue N content in *D. flexuosa* standardised grass plants exposed along a NH_3 gradient over a *Calluna vulgaris- Eriophorum vaginatum* dominated mire at Whim Moss. Mean ± 1 SE.

The tissue N content amongst replicates did not vary much, with the exception of those plants exposed to the highest NH_3 concentration (69 µg m^{-3}). The reason for this variation is unclear, although it suggests some form of differential uptake between the pots of *D. flexuosa*.

By ignoring the 69µg m^{-3} point, the relationship for NH_4-N concentration is rather similar to that for tissue N content. As with % N content, there was a relationship with foliar soluble NH_4^+ concentration increasing with atmospheric NH_3 concentration, but this was rather scattered (R^2=0.563). Again, as with tissue N content, the most uncertain data (largest error bar) was found for the site with 69 µg m^{-3}, reflecting a high variation in response at this high exposure level (Figure 5.24).

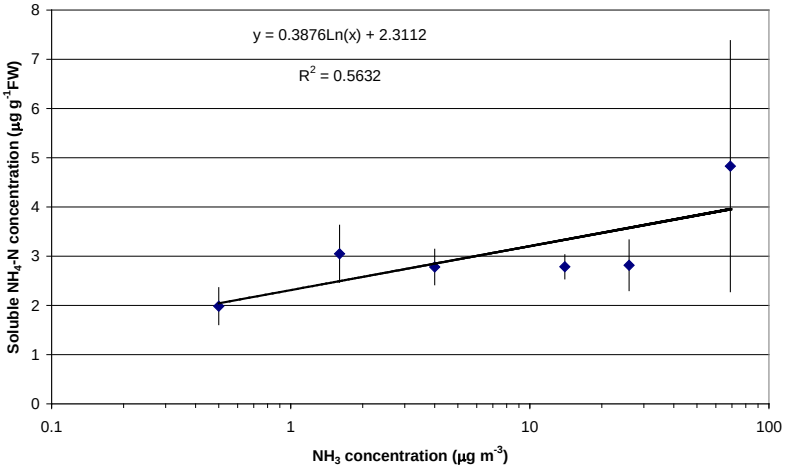


Figure 5.24. The mean soluble NH_4-N concentration in *D. flexuosa* standardised grass plants exposed along a NH_3 gradient over a *Calluna vulgaris- Eriophorum vaginatum* dominated mire at Whim Moss. Mean ± 1 SE.

5.3.10. Wet N treatments: Biomass determination

The *D. flexuosa* plants responded positively to the wet N treatments by showing a general increase in biomass accumulation in all the N form treatments compared with the control treatment (Figure 5.25). However, there were only significant differences between the control and the 32 kg N ha^{-1} y^{-1} NO_3^- and 64 kg N ha^{-1} y^{-1} but not the NH_4^+ 32 or 64 kg N ha^{-1} y^{-1} treatments. Although, the NO_3^- (64 kg N ha^{-1} y^{-1}) treatment had a much higher mean biomass it was not significantly different from its NH_4^+ (64 kg N ha^{-1} y^{-1}) equivalent. Although there were observed trends, the non-significance could be the result of the small sample size. The biomass accumulation, biochemical foliar analysis and total N inventory for Whim Moss standardised grass transplant study are summarised in Table 5.6.

Table 5.6. Summary table of the wet N deposition results of the standardised grass biomonitors study at Whim Moss, Scottish Borders.

Treatment	Annual N dep.	Mean Biomass (g)	% N content	Sol NH4-N conc.	Total N inventory
Control	10	0.55	0.96	7.08	5.3
NH_4^+ dep.	32	1.44	1.06	23.2	15.1
NO_3 –dep.	32	1.88	1.01	5.03	18.9
NH_4 dep.	64	1.26	1.10	8.29	13.9
NO_3^- dep.	64	2.68	0.98	5.46	26.2
Effect of NO_3^- (32 vs. 64)		p=0.399	p=0.801	p=0.224	p=0.371
Effect of NH_4^+ (32 vs. 64)		p= 0.601	p=0.500	p=0.354	p=0.726
Effect of NH_4^+ vs. NO_3^- (at 32)		p=0.369	p=0.683	p=0.204	p=0.310
Effect of NH_4^+ vs. NO_3^- (at 64)		p=0.150	p=0.106	p=0.573	p=0.196

Statistical analysis using Analysis of variance: n=3.

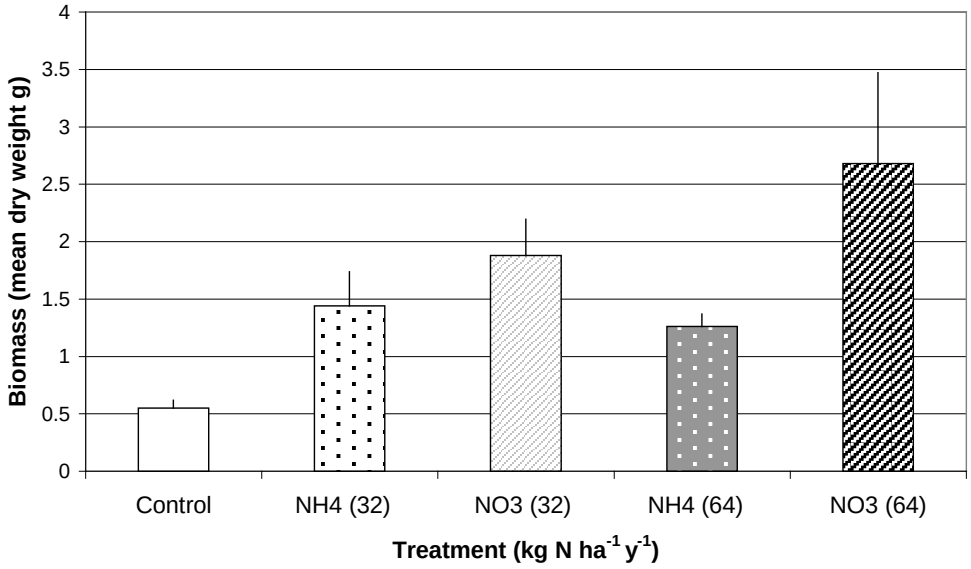


Figure 5.25. *D. flexuosa* biomass after exposure to 12 weeks of wet N treatments at Whim Moss manipulation facility. Mean ±1 SE.

5.3.11. Wet N treatments tissue N content (% dry weight) and NH_4-N concentration

There was no significant effect of either N form on the uptake of N as shown in the tissue N content (p=0.525) (Figure 5.26). The lack of significant treatment effect on the tissue N content may be the result of growth dilution in both the NO_3^- and NH_4^+: 32 and 64 kg N ha^{-1} y^{-1} treatments. Both the 32 and 64 NH_4^+ treatments had a higher tissue N content than the corresponding NO_3^- treatments. This is consistent with the effects of the different N forms shown for the pleurocarpous moss *H. jutlandicum* (Section 6).

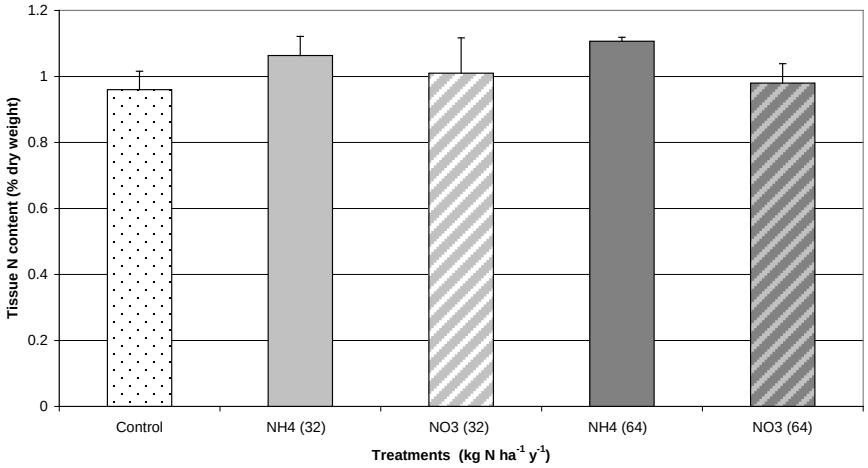


Figure 5.26. Total mean tissue N content of *D. flexuosa* tillers exposed to different N forms of wet N treatments at Whim Moss. Mean ± 1 SE.

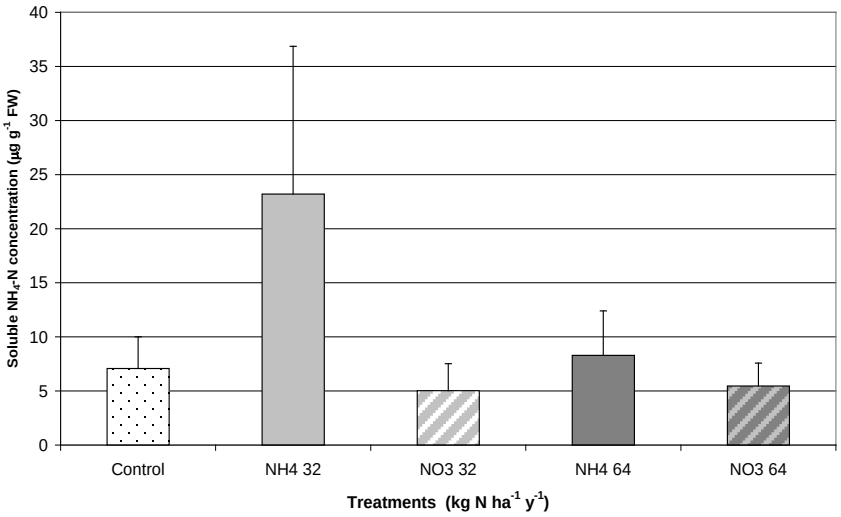


Figure 5.27. Total mean soluble NH_4-N concentration (µg g^{-1} FW) *in D. flexuosa* tillers exposed to different N forms of wet N treatments at Whim Moss. Mean ± 1 SE.

The increase in soluble NH_4-N concentration in the NH_4^+ treatment compared to the control and the NO_3^- treatments is not unexpected as this was the target N species in the chemical analysis (Figure 5.27). However, the difference between the N forms in both the 32 and 64 kg N ha^{-1} y^{-1} treatments was not significant. The large mean concentration in the 32 kg N ha^{-1} y^{-1} NH_4^+ treatment was not significantly different from the other treatments due to the large variation between the replicate samples.

5.3.12. Wet N treatments N Inventory

The total above ground foliar N inventory (Figure 5.28) shows that all N treatments responded to the additional wet N inputs. All treatments had a significant increase in foliar N inventory when compared to the control. Although, there was no significant difference between the N forms at the 32 kg N ha^{-1} y^{-1} level, there was at the 64 kg N ha^{-1} y^{-1} (P<0.05). This would suggest that *D. flexuosa* might prefer NO_3^- as an N source rather than NH_4^+. However, the large increase in N mg/pot in the NO_3^- treatments is predominantly driven by the large increase in biomass as the tissue N content was higher in the NH_4^+ treatments.

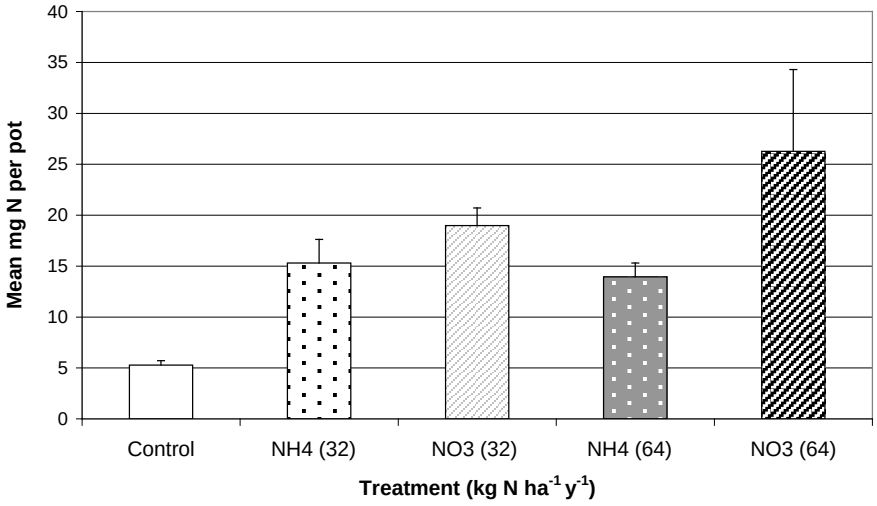


Figure 5.28. Total above ground N inventory for *D. flexuosa* standardised plants exposed to different N forms at Whim Moss N manipulation facility. Mean ±1 SE.

5.3.13. Comparison between soluble NH_4-N concentration and tissue N content (% dry weight) for Piddles Wood, Happendon Wood, Auchencorth Moss and Bowbeat Hill.

Comparison between the two biochemical methods (tissue N content and soluble NH_4-N concentration) across the four sites (Piddles Wood, Happendon Wood, Auchencorth Moss and Bowbeat Hill) shows a reasonable relationship between them (Figure 5.29). The data plotted in Figure 5.28 are for all the intensive sites but excludes the Whim moss manipulation study data. There was a reasonably good relationship between the two foliar N methods (R^2= 0.759). This would suggest that both methods could be used to assess the impacts of ammonia or N deposition on grass biomonitors. However, the relationship between tissue N and NH_4-N concentration was poor for the *D. flexuosa* at Whim Moss (R^2=0.102) data not shown.

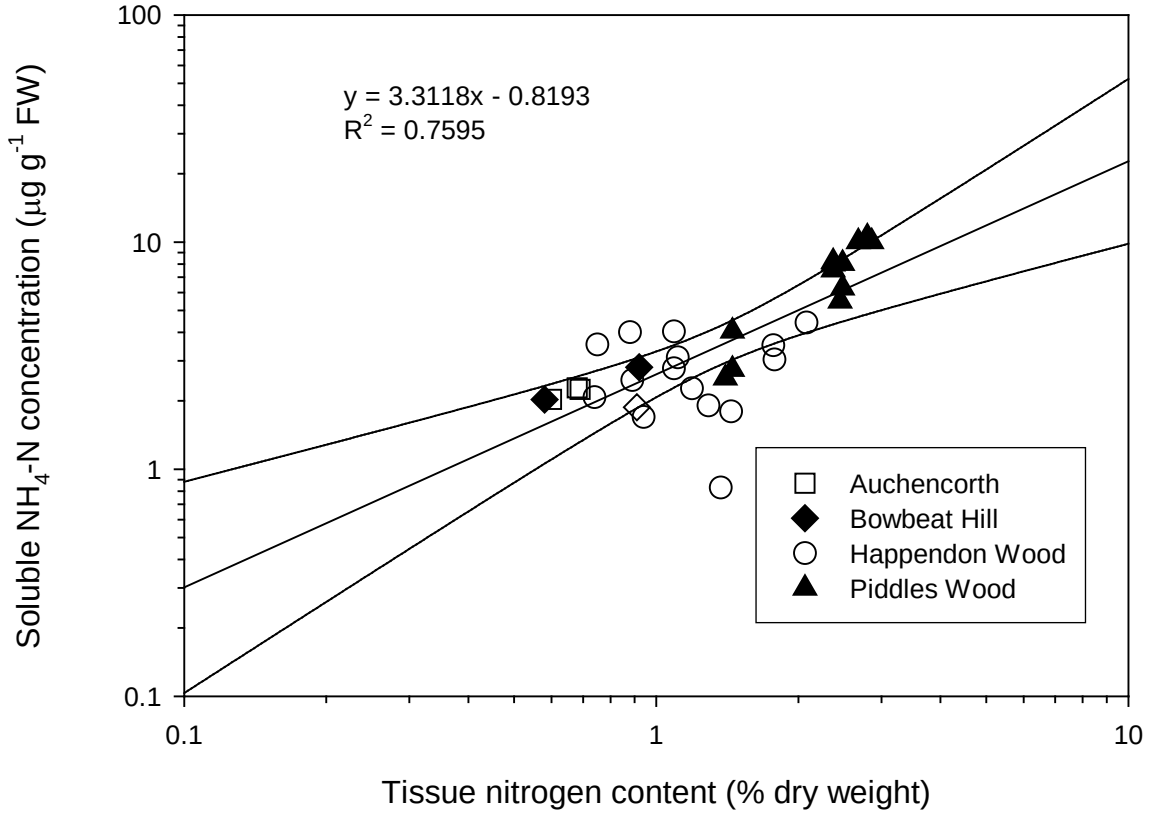


Figure 5.29. Comparison of the NH_4-N concentrations (µg g^{-1} FW) and tissue N content (% N dry weight) in *D. flexuosa* for Auchencorth, Bowbeat, Happendon Wood (M74) and Piddles Wood) R^2=0.7595 and 95% confidence intervals.

5.4. Discussion

Due to the relatively low total N inputs at some of the selected intensive sites the exposure period of the study required to be at least 3 months in duration. Therefore, the standardised grass species selected for this study had to be a slower growing species in order to last the duration of the exposure period and had to be able to withstand a greater range of conditions than the *Lolium* spp., which has been applied in previous studies (Sutton *et al*. 2004a, Sommer and Jenson 1991).

It is believed that this was the first application of *Deschampsia flexuosa* as a standardised grass biomonitor of enhanced N. Although, *D. flexuosa* performed well under field conditions at Piddles Wood, Happendon Wood and Whim Moss, it was not as easy to propagate from seed compared to *Lolium* species. If this species were to be used extensively

as a biomonitor there would then be a requirement for the development of an improved seed germination method.

The main benefit of using a slower growing species such as *D. flexuosa* is to allow the exposure period to be extended at sites with a low N imput. It is believed that *D. flexuosa* fits this requirement, even though the conditions at Auchencorth Moss and Bowbeat Hill appeared to directly impair the growth of the *D. flexuosa*. This use of standardised grass transplants at sites exposed to diffuse N inputs offers the potential for providing living N bionindicators to assess potential site impacts; however a larger exposure period and greater sample size would improve the use of this method.

The data presented in the current report shows that *D. flexuosa* was able to pick up signals of N inputs at Piddles Wood in biomass accumulation, tissue N content and soluble NH_4-N concentration. The increase in biomass accumulation in *D. flexuosa* at the site closest to the poultry unit at Piddles Wood was approximately 65% smaller than for *Lolium perenne*, which was used at a similar poultry unit in Sutton *et al.* 2004a.

The tissue N content signal in *Deschampsia flexuosa* also appeared to be approximately 30% smaller at Piddles Wood than that found for *Lolium perenne* at a poultry unit with smaller NH_3 concentrations (Sutton *et al*. 2004a). However, this could be due to species difference in partitioning nitrogen.

At Happendon Wood, signals were seen in biomass accumulation and tissue N content along the NO_2 concentration gradient and with total N deposition but not with NH_3 concentration. The decrease in biomass close to the source (M74) was in the contrary direction to that found for both Piddles Wood and Whim Moss. As there are strong N sources at these two sites compared to Happendon Wood, the difference in biomass response at Happendon Wood cannot be directly attributed to N deposition. It would indicate that other factors are dominating biomass. No signals were seen in the soluble NH_4-N concentrations with any of the N soures. The small number of grass plants exposed in the current study did not show any strong signal with N inputs at the moorland sites. A more intensive study is required at remote sites with long-range N inputs to determine if standardised grass transplants are a suitable method for the detection of N impacts on these types of site.

The best relationships with biomass, tissue N content and NH_4-N concentration were found at the agricultural point source site (Piddles Wood). There were strong linear relationships with the log distance from the poultry unit, between atmospheric NH_3 concentration, total N deposition and biomass and for both foliar N measurement methods along the transect. The large above-ground vegetation growth rate could have caused growth dilution of the foliar N concentrations, with the measured concentrations being an underestimate of the total N uptake.

The increase in tissue N contents, soluble NH_4-N concentrations and biomass in the *D. flexuosa* plants along the NH_3 gradient at Piddles Wood showed that N was directly impacting on the ground vegetation at this site. The results also indicate that the long-term integrity of the Piddles Wood SSSI, including an increasingly larger area surrounding the poultry unit, could be under direct threat from accumulated N deposition. As with Sutton *et al*. (2004a) the N inventory provided a better relationship than tissue N content and biomass, which suggests that the affects of different levels of shading were cancelled out in the N inventory.

There was a significant reduction in biomass and an increase in foliar N content at the sites closest to the M74 motorway at Happendon Wood. However, it is uncertain whether it was growth inhibition due to other traffic-based pollutants or due to variations in shading intensity. The NO_2 concentrations were approximately 50% lower than those reported in a

case study on the M62 motorway reported by Bignal *et al*. (2004). It is unclear why there was no response in NH_4-N concentration in the standardised plants along the gradient away from the motorway. This could indicate that % N is a much better method to employ at this type of site. In contrast to Piddles Wood, the relationship with N inventory and N inputs were much poorer at Happendon indicating that the N inventory is a better indicator at sites with larger N inputs.

It is unclear why there was little difference between Auchencorth Moss and Bowbeat Hill in the measured biomass and foliar N concentrations even though Bowbeat Hill has approximately double the N deposition.

The results of the wet N treatments at Whim Moss show there was an impact on biomass, which was dependent on N form, with NO_3^- treatments (32 and 64 kg N ha^{-1} y^{-1}) resulting in a significant increase in biomass accumulation rate of *D. flexuosa* compared with the control treatment. This increase was not observed in the NH_4^+ treatments. The % N was slightly higher in the NH_4^+ treatments than for the NO_3^- treatments, indicating a greater growth dilution from NO_3^- and more accumulation in the NH_4^+ treatments. It could also indicate an uptake by the leaves as NH_4^+, whereas NO_3^- is not normally absorbed by the above ground plant material.

The lack of N response in either of the foliar N methods indicates that the N inputs (2.5, 5, 10 kg N ha^{-1} y^{-1}) respectively for the three treatments over the 3 months exposure period were insufficient to impact on the *D. flexuosa* grass biomonitors. This suggests that a longer exposure period would be required to detect any N impact using *D. flexuosa* as standardised N grass biomonitors.

The results indicate that any potential increase in N deposition at a specific site could be detected in the foliar N uptake of standardised *D. flexuosa* plants but it would require a N input of >32 kg N ha^{-1} y^{-1} to detect a difference over a three month exposure period.

It is difficult to compare inter site variation in biomass, total N content and NH_4-N concentrations for the 5 intensive sites because of the large differences in habitat, altitude, longitude, meteorological conditions and N strength and source attribution. However, the results would indicate that there is no real advantage in using *D. flexuosa* at agricultural point source sites compared to *Lolium* species.

It was interesting that there was a differential response between the % N and soluble methods at Happendon Wood, with % N detecting an N input signal. This indicates the importance of selection of an appropriate method for a particular habitat. The importance of the use N inventory in overcoming shading problems was observed as it was in Sutton *et al* (2004a) with *L. perenne*.

5.5. Conclusions

- The measurements show that *D. flexuosa* could be used effectively as standardised grass species for N biomonitoring, especially at sites with a defined point source.

- The applied methodology using a wicking system linked to reservoirs of water worked well. The grass N biomonitors only required minimal management over the three months exposure period with a visit every 10-14 days to check on the grass biomonitors status.
- The method was relatively cheap to use and does not require specialist equipment (equipment cost per six plant tray was ~ £25). There would be an analytical cost of ~ £10-20 per sample for tissue N content and soluble NH_4-N concentration.
- The grass biomonitors method does not require specialist expertise but some knowledge of potential grass biomonitor species and their method of propagation are essential.
- However, the germination and propagation of the *D. flexuosa* seed was not as straight forward as for *Lolium* spp. Further work to improve the germination rate/propagation techniques for this species would be required.
- The biochemical methods (tissue N content and soluble NH_4-N concentration) and biomass accumulation were able to detect N inputs at the agricultural point source site at Piddles Wood.
- At the motorway site signals in % N and biomass were found along the gradient away from the M74 motorway. However, no signal was found for soluble NH_4-N concentration.
- The NO_3-N treatments increased but not significantly the above ground biomass compared to the equivalent NH_4-N treatment at Whim Moss.
- The wet N input levels during the 3-month exposure period at Whim Moss (2-10 kg N ha^{-1} y^{-1}) were insufficient to detect a significant signal in the biochemical methods employed. However, the tissue N contents and the soluble NH_4-N concentrations were greater in the NH_4-N treatments at both 32 and 64 kg N $ha^{-}1$ y^{-1} than the corresponding NO_3-N treatments. This would indicate that there is an effect of N form on foliar N uptake with increases per unit N being generally greater for NH_4-N than NO_3^{-}N. This is consistent with the results for *H. jutlandicum* (Section 5).
- The application of standardised grass N biomonitors at sites with a diffuse N source attribution and/or lower total N deposition values (14 and 25 kg N ha^{-1} y^{-1}) is not an effective method to monitor potential N impacts over a short period (i.e. 0-3 months).
- The effective use of grass biomonitors such as *D. flexuosa* would probably require a longer exposure period (6-12 months) to detect potential N impacts at long-range N input sites.
- Additional work is required to identify alternative slow growing grass species, which could be used as standardised grass biomonitors at sites exposed to relatively low N inputs and therefore would require plants to be exposed for period in excess of 3 months.

6. Impacts of N on pleurocarpous mosses at the intensive sites

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6.1. Introduction

Mosses have previously been used as bioindicators of gaseous pollutants along a gradient of ammonia concentration from point sources such as poultry units (Pitcairn *et al.* 2003). There have been a limited number of field studies assessing the impacts of vehicular emissions using a transect approach and these have studied single species, habitats and plant-insect interactions. Effects on mosses in a reciprocal transplant study have shown increased N concentrations in *Dicranum scoparium* closer to the motorway (Bignal *et al*. 2004). The agricultural and road studies to date have been with a defined and easily measurable source of N deposition. The use of mosses as bioindicators in areas with an undefined low-level N source (precipitation and/or orographic), such as upland areas have not been widely tested.

The aim of this part of the study was to examine the use of mosses as bioindicators, thus utilising their potential as a tool to improve detection of early N deposition effects on a range of habitat types, exposed to both localised and diffuse source of N concentration and deposition, which therefore could be applied to condition assessment and common standards monitoring of statutory nature sites.

Five extensive sites were selected to refine and test a range of potential bioindicator methods including the use of pleurocarpous moss total tissue N content (as expressed as %) and soluble NH_4-N concentration (see section 3 for site description). The sites were selected in order to represent the different types of localised and diffuse N sources under a range of habitat types. The five sites were a) Piddles Wood, a designated SSSI lowland woodland situated adjacent to a localised NH_3 point source (intensive agricultural poultry unit); b) Happendon Wood situated close to a diffuse point source (the M74 motorway) dominated by NO_X and NH_3 emissions; c) Bowbeat Hill, a rural upland site with no localised source but dominated by wet deposition; and d) Auchencorth Moss, an equivalent low elevation site dominated by wet deposition. In additional the experimental controlled-release site at Whim Moss was also used in this study to examine the single species of pleurocarpus moss, *Hypnum jutlandicum* exposed to predetermined concentration of gaseous NH_3 and two single forms of wet N deposition (NH_4^+ or NO_3^-) through enriched precipitation. The site at Whim Moss field facility is a long-term study examining the effects of both wet and gaseous N on M19a – a mire vegetation. The Whim Moss site and automated N release systems are described in more detail under Section 3.

Pleurocarpous mosses were collected at each of the 5 extensive sites and analysed for tissue N content and soluble NH_4-N concentration. At Piddles Wood and Happendon Wood the moss species selected were those most frequently found at the different distances along the transects away from either the poultry unit or the M74 motorway. The moss species collected at Auchencorth and Bowbeat Hill was *Pleurozium schreberi*, the most abundant species at these sites. At Whim Moss, *H. jutlandicum* was selected as it was the most frequently occurring moss in both the wet and dry N deposition treatment plots.

6.2. Methods

6.2.1. Preparation of moss samples for tissue N content and soluble NH_4-N concentration analysis

The following procedures were applied to all collected pleurocarpous mosses in the intensive study sites described in this section and the extensive UK study (described in Section 9).

Sorting and cleaning procedures for moss samples (CEH laboratory*)*

Disposable gloves were used throughout the procedure, to reduce any cross-contamination of free N.

- The collected mosses were stored in polythene bags at 4º C until ready for species identification checking and sorting/cleaning.
- The moss samples were checked for correct species identification prior to sorting and any unwanted species were discarded from the sample.
- From the remaining sample approximately 3-4 g (FW) of the green, clean shoots were selected (only the top 2-3 cm of the individual moss shoots were used)
- The selected moss shoots were rinsed with de-ionised water to remove any possible surface N contamination, soil particles or debris.
- Excess water was removed from the sample by shaking in a plastic sieve.
- At this point the cleaned samples were separated into two duplicate samples. One sample was used for determing tissue N content (expressed as % dry weight) and the other for soluble NH_4-N concentration.

Sample preparation for tissue N content (% dry weight)

- After separation the samples for % N determination were placed in a well marked paper bags and dried for 48 hours at 70 ^{0}C.
- The samples were then ground to a fine powder using a ball mill and placed in a 3 ml glass vial.
- The sample was analysed for % N content by CN analysis using a Vario-EL elemental Analyser.

Sample preparation and analysis for soluble NH_4-N concentration

- The second samples were analysed for soluble NH_4-N concentration using methodology described in Appendix I.

6.3. Results

6.3.1. Moss sampling at Piddles Wood

Three replicate samples were taken of the two most frequently occurring moss species, *Eurynchium praelongum* and *Eurynchium striatum* at each of the 5 distances away from the poultry unit on 27 April 2004. *E. striatum* was present at all 5 distances but *E. praelongum* was absent at the background ambient site at 250 m from the poultry unit (See Section 3.2.2 for site description).

6.3.2. Results at Piddles Wood

The tissue N content and soluble NH_4-N concentration of the two species, *E. praelongum* and *E. striatum* declined sharply with increasing log distance from the poultry unit (Figure 6.1 and Figure 6.2 respectively). Although the relationship between soluble NH_4-N concentration and distance for *E. praelongum* was not as strong as that found for total N content ($R^2 = 0.854$), while that for *E. striatum* was slightly better than for tissue N ($R^2 = 0.983$). The response to N deposition in the transect was much larger for soluble NH_4-N than for % N: Overall, tissue % N content increased by a factor of 2.7, while soluble NH_4-N content increased by a factor of 20 for *E. praelongum* and a factor of 40 for *E. striatum*, the closer to the source.

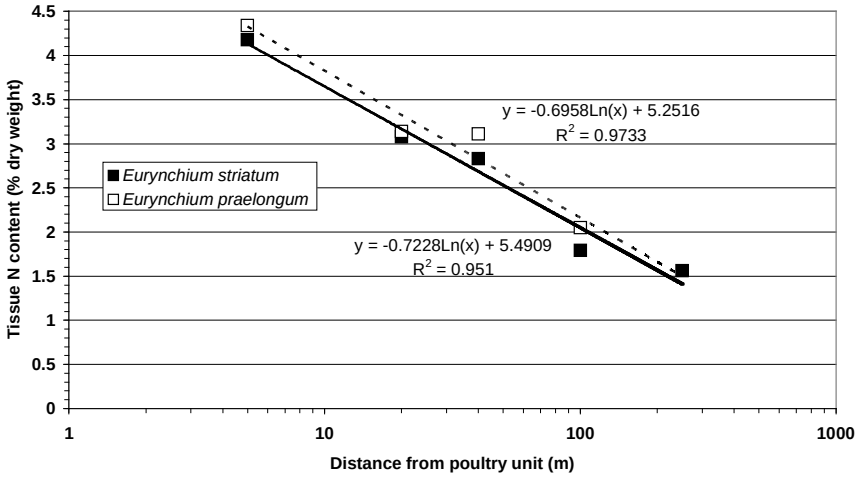

Figure 6.1. The relationship between the tissue N content of two pleurocarpous mosses and distance (m) from the poultry unit at Piddles Wood, Dorset.

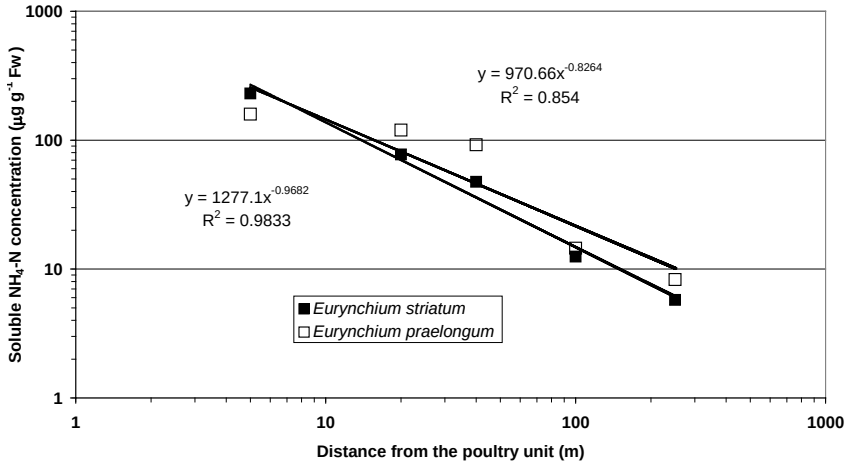

Figure 6.2. The relationship between the soluble NH_4-N concentration (µg g^{-1} FW) of two pleurocarpous mosses and distance (m) from the poultry unit at Piddles Wood, Dorset.

When plotted against log atmospheric NH_3 concentration (Figures 6.3 and 6.4) and N deposition (Figures 6.5 and 6.6), both soluble NH_4-N and total N content increased increasing with increasing exposure.

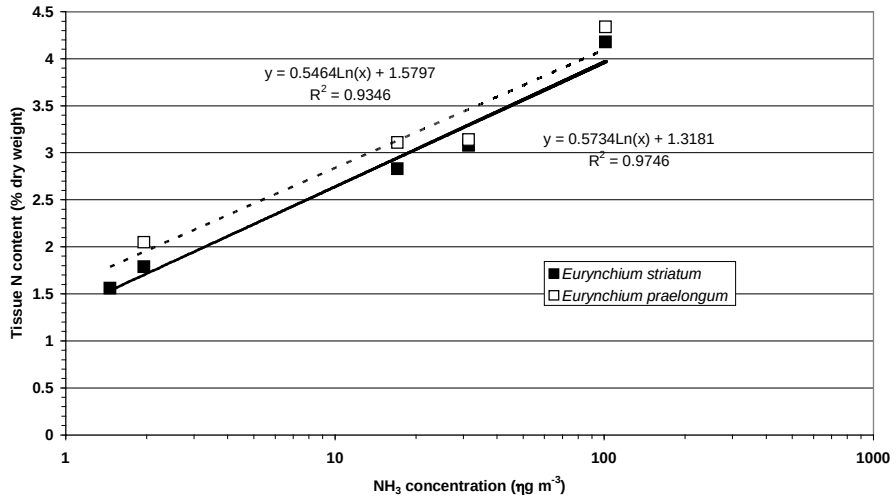

Figure 6.3. The relationship between the tissue N content of two pleurocarpous mosses and NH_3 concentration at Piddles Wood, Dorset.

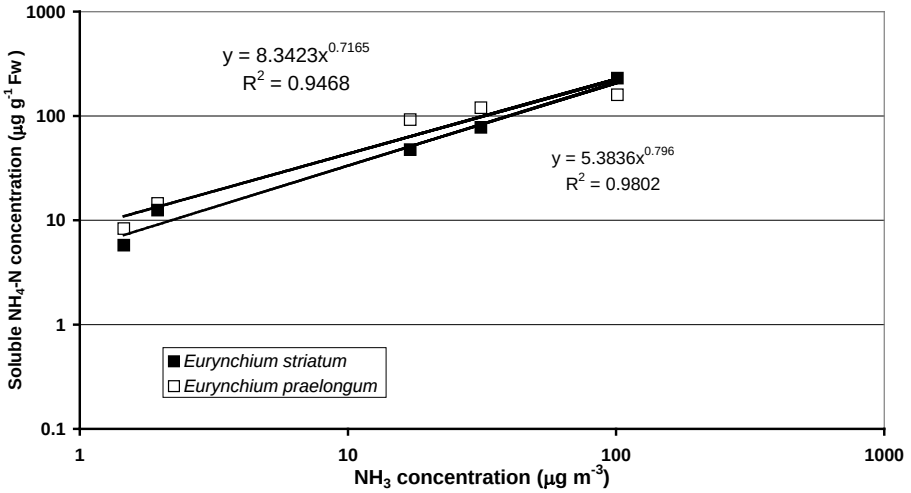

Figure 6.4. The relationship between exposure to atmospheric NH_3 concentrations and soluble NH_4-N concentration in moss tissue along a defined NH_3 gradient transect from the poultry unit at Piddles Wood, Dorset.

Although, tissue N content increased linearly with the log of N deposition in *E. praelongum* (Figure 6.5) the response of soluble NH_4-N concentration to increased N deposition resulted in a curved relationship (Figure 6.6). However, this type of response was not found for *Eurynchium striatum*.

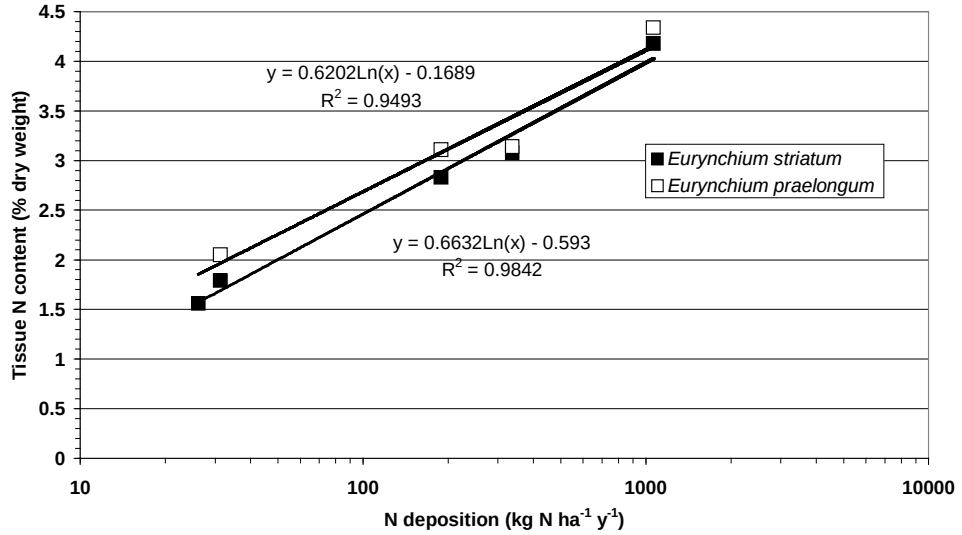

Figure 6.5. The relationship between tissue N content for two pleurocarpous mosses and total N deposition at Piddles Wood, Dorset.

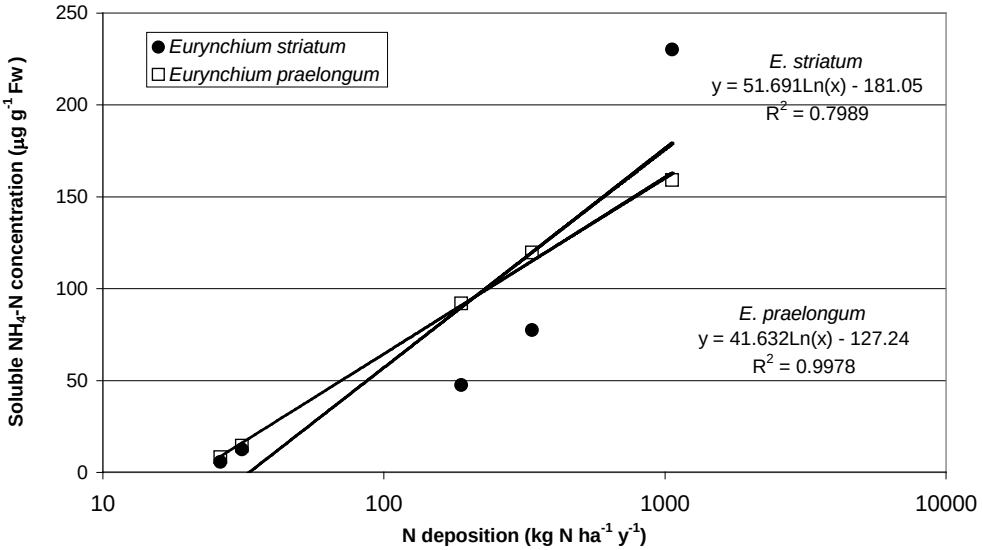

Figure 6.6. The relationship between soluble NH_4-N concentration in the tissue of two pleurocarpous mosses and total N deposition at Piddles Wood, Dorset.

6.3.3. Moss sampling at M74 motorway site at Happendon Wood

At each site, four pleurocarpous moss species were collected at six distances along the 250 m transect away from the M74 motorway (*Rhytidiadelphus squarrosus*, *Eurhynchium praelongum, Scleropodium purum* and *Rhytidiadelphus loreus*). Of the four species, only *R. squarrosus* was represented at all distances along the pollution gradient.

6.3.4. Results of moss sampling at M74 motorway site at Happendon Wood

The tissue N content in *R. squarrosus* decreased significantly with distance from the M74 (Figure 6.7) from 2.9% N at 10 m from the motorway to 1.06% N at a distance of 250 m. It was interesting that E. *praelongum* was only found close to the M74 (N deposition ~70 kg N ha^{-1} y^{-1}). This species was also present at the sites closest to the poultry unit at Piddles Wood, which would suggest that this species has a higher affinity for habitats with enhanced N

inputs. *E. praelongum* is predominately a woodland species and likes deeper shade than many other species. The regression lines plotted in Figures 6.7–6.12 are for the *R. squarrosus* data only.

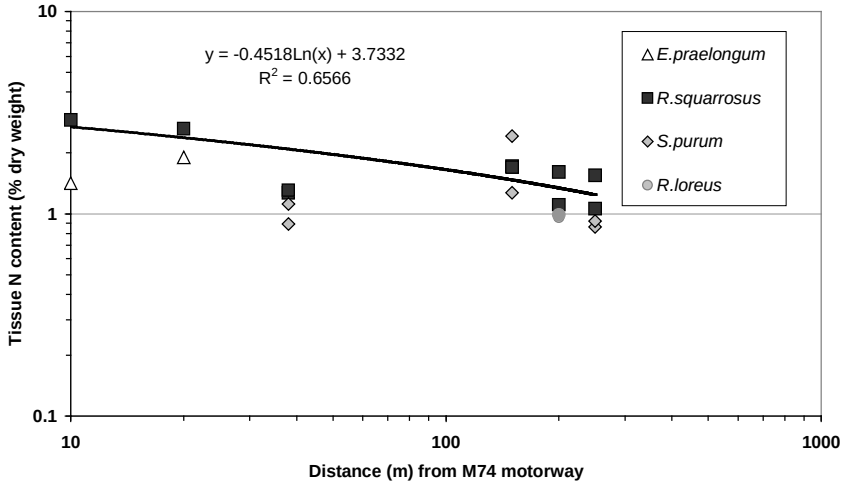

Figure 6.7. The tissue N content of four pleurocarpous moss species collected at different distances away from the M74 motorway at Happendon Wood, south Lanarkshire.

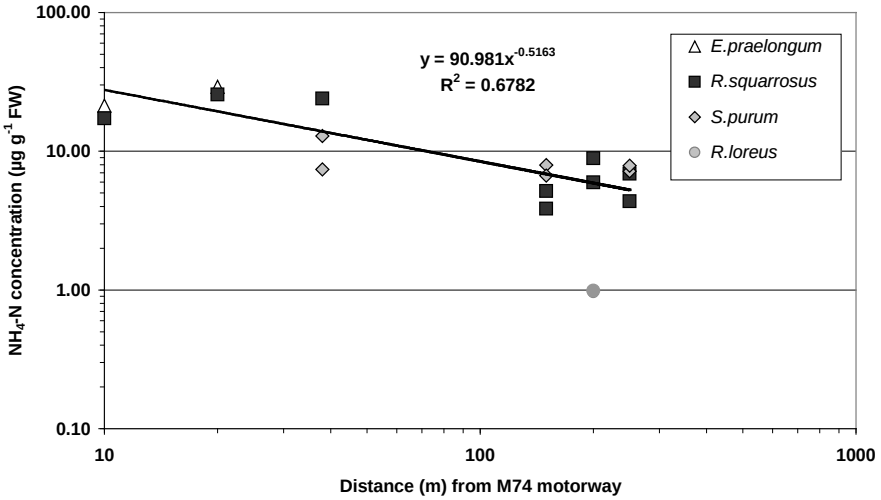

Figure 6.8. The soluble NH_4-N concentration of four pleurocarpous moss species collected at different distances away from the M74 motorway at Happendon Wood, south Lanarkshire.

A similar log-log relationship was found for soluble NH_4-N concentration and distance from the motorway. Both *R. squarrosus* and *E. praelongum* exhibited the largest soluble NH_4-N concentrations at the site closest to the M74. However, *E. praelongum* was found at the two closest sites to the motorway. The soluble NH_4-N concentration ranged from 30 μg g^{-1} FW closest to the M74 to 4 μg g^{-1} FW at the 250 m distance in *R. squarrosus*, the only species found across the full transect (Figure 6.8). Hence, across the transect at Happendon Wood, tissue N content (in *R. squarrosus*) varied by a factor of 3, while soluble NH_4-N concentration varied by a factor of 6, consistent with the larger relative response to increased N for soluble NH_4-N concentration seen at Piddles Wood. It must be noted that the dominant N form at the two sites differed with NO_2 at Happendon Woodland and NH_3 at Piddles Wood.

There was a much poorer relationship with tissue N content and soluble NH_4-N concentration with atmospheric NH_3 concentration than was found for distance (Figure 6.9 and 6.10 respectively). This is consistent with the fact that N deposition at this site receives a larger contribution from NO_2 (Section 3).

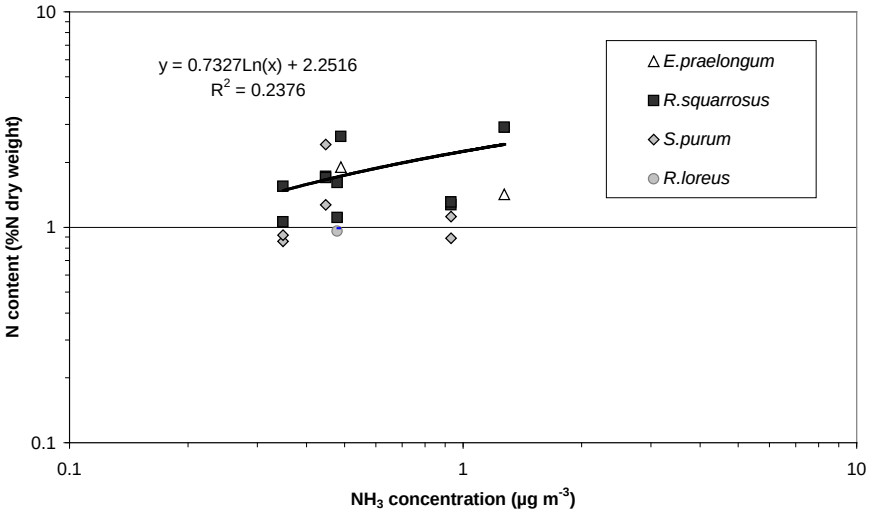

Figure 6.9. The tissue N content of four pleurocarpous moss species measured at different distances from the M74 motorway at Happendon Wood, south Lanarkshire.

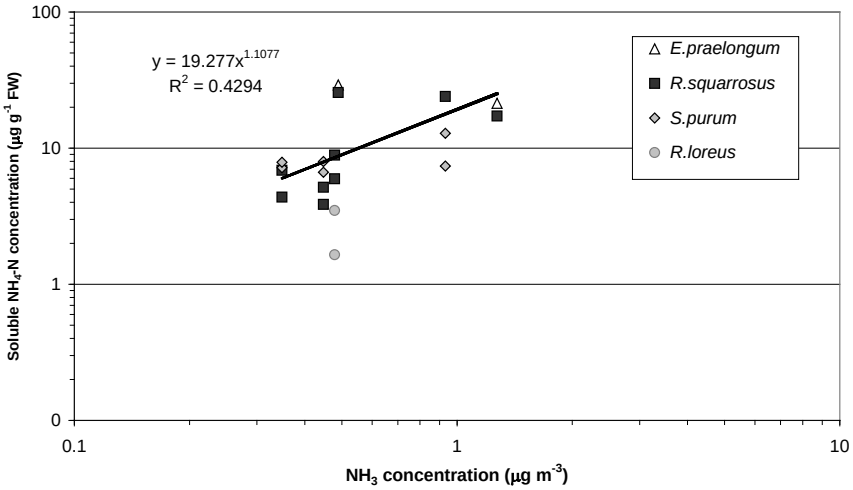

Figure 6.10. The soluble NH_4-N concentration of four pleurocarpous moss species measured at different distances from the M74 motorway at Happendon Wood, south Lanarkshire.

The stronger relationship was with the soluble NH_4-N concentration, indicating that this method is more sensitive than the N content.

There was no significant relationship between tissue N content and total N deposition for the moss *R. squarossus* (Figure 6.11). However, soluble NH_4-N concentration (Figure 6.12) did show a linear relationship between exposure, thus indicating the difference in responses obtained between the two methods employed, suggesting soluble NH_4-N method is a more sensitive for the varying N inputs at this site.

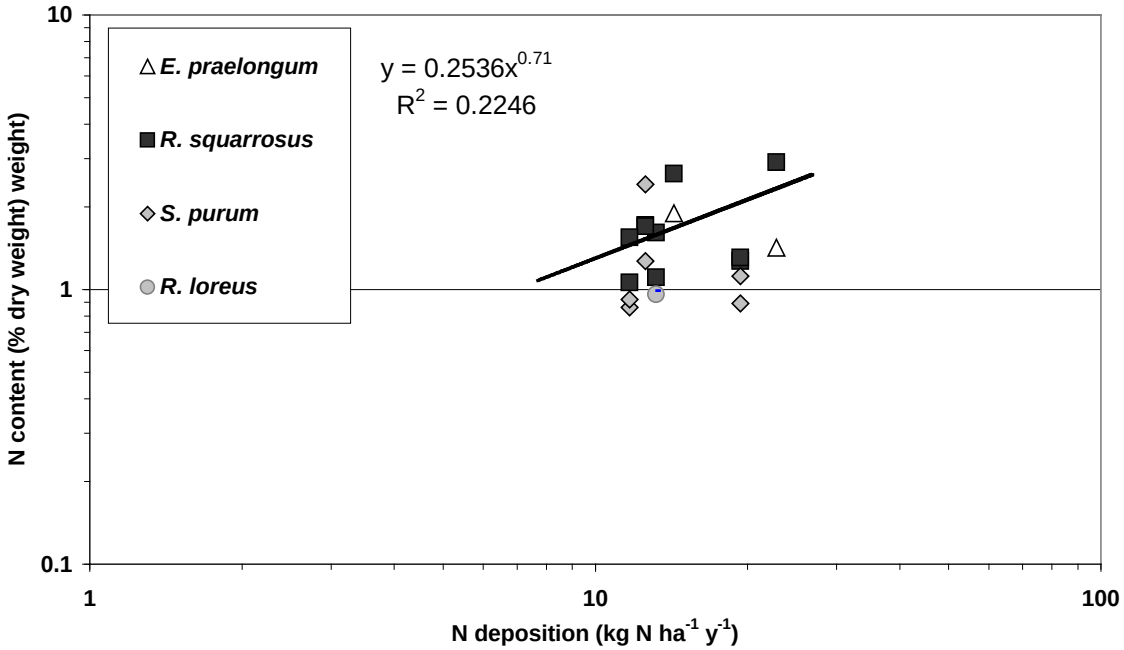

Figure 6.11. The tissue N content of four pleurocarpous moss species (collected at different distances away from the M74 motorway plotted against total N deposition at Happendon Wood, south Lanarkshire.

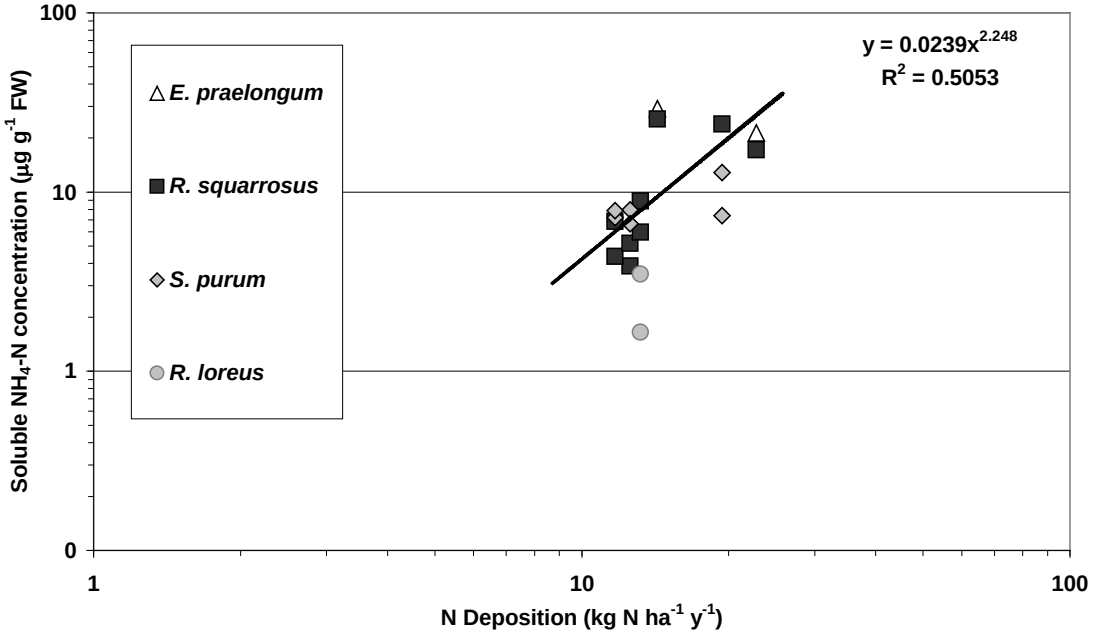

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Figure 6.12. The soluble NH_4-N concentration of four pleurocarpous moss species (collected at different distances away from the M74 motorway plotted against total N deposition at Happendon wood, south Lanarkshire.

The pleurocarpous moss, *R. squarrosus* exhibited strong relationships between both tissue N content and soluble NH_4-N concentration and the log NO_2 exposure concentrations (Figure 6.13). This suggests that the NO_2 concentrations from the M74 are impacting on *R. squarrosus*.

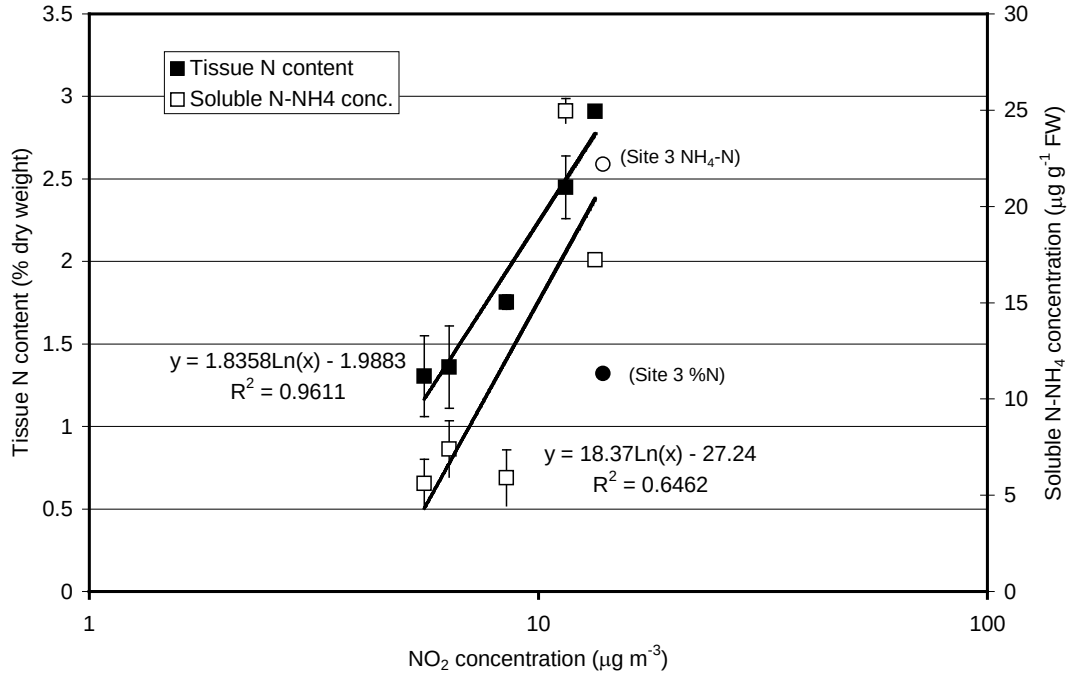

Figure 6.13. The impact of NO_2 concentration on tissue N content and soluble NH_4-N concentration of *Rhytidiadelphus. squarrosus* at Happendon Wood.

6.4. Relationship between total tissue N content of mosses and soluble NH_4-N concentration at the intensive sites Piddles Wood and Happendon Wood

There is a strong relationship ($R^2 = 0.965$) between tissue N content and soluble NH_4-N concentration for the two species of pleurocarpous moss sampled along the NH_3 gradient at Piddles Wood (Figure 6.14). The two mosses sampled *E. praelongum* and *E. striatum* are known woodland species with *E. praelongum* often found in areas close to point sources of NH_3 (Pitcairn *et al.* 2003). Overall, foliar NH_4-N concentration appeared to be a more sensitive parameter with NH_3 concentration at Piddles Wood varying by over a factor of ~30 compared with total foliar N, which varied by a factor of 2.7.

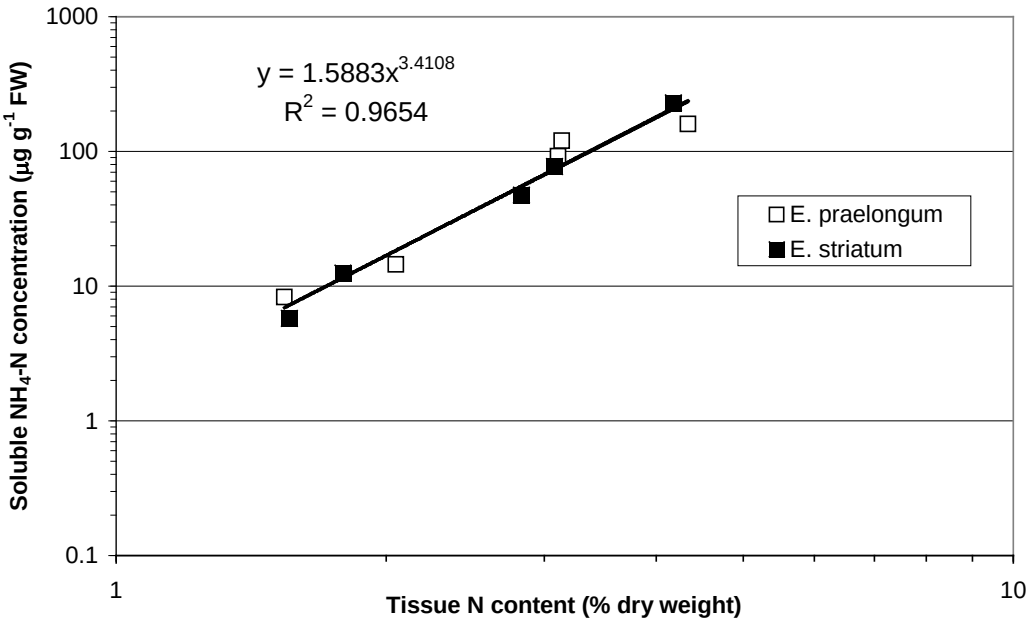

Figure 6.14. The relationship between the tissue N content and soluble NH_4-N concentration for two pleurocarpous mosses (*Eurhynchium praelongum* and *Eurhynchium striatum*) at the poultry unit at Piddles Wood, Dorset.

By contrast to the excellent relationship at Piddles Wood, there was a poor relationship between tissue N concentration and soluble NH_4-N concentration for the pleurocarpous mosses sampled at Happendon Wood (R^2 =0.239: Figure 6.15). The difference in the relationship between the two sites could be due to only two mosses being used at Piddles Wood compared to four at Happendon Wood. Additionally, the mosses (*E. praelongum* and *E. striatum*) sampled at Piddles Wood had a much higher tissue N contents and soluble NH_4-N concentrations than for these two species at Happendon Wood (Table 6.1).

Table 6.1. Comparison of total N concentration and NH_4-N concentration (µg g^{-1} FW) for two pleurocarpous mosses at Piddles Wood and Happendon Wood.

	E. praelongum			***E. striatum***		
	N deposition (kg N ha^{-1} y^{-1})	**Tissue N content (% N)**	**Soluble NH_4-N concentration (µg g^{-1} FW)**	**N deposition (kg N ha^{-1} y^{-1})**	**Tissue N content (% N)**	**Soluble NH_4-N concentration (µg g^{-1} FW)**
Piddles Wood	1061	4.4	149	1061	4.2	230
Happendon Wood	22	1.42	21.3	14.3	1.9	16.9

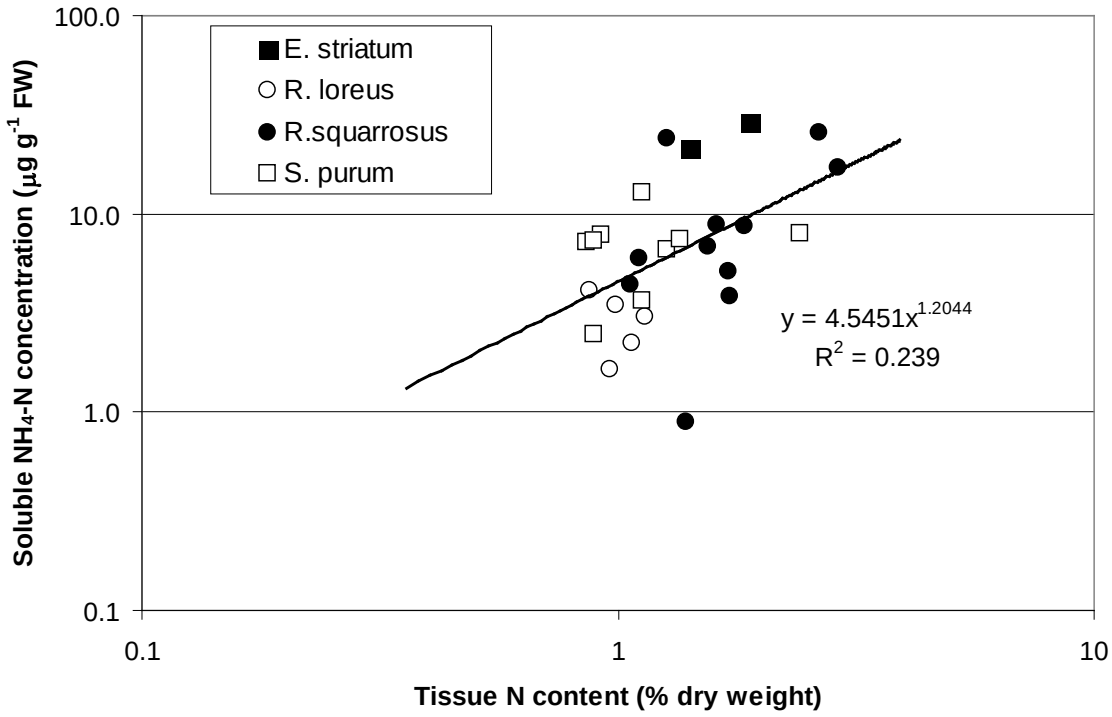

Figure 6.15. The relationship between the tissue N content and soluble NH_4-N concentration for four pleurocarpous moss species (*R. squarrosus*, *E. praelongum*, *S. purum* and *R. loreus)* at Happendon Wood, south Lanarkshire.

6.5. Auchencorth moss and Bowbeat Hill

The only moss species collected at both sites was *Pleurozium schreberi*. The tissue N content of *P. schreberi* was higher at Auchencorth Moss (mean 0.88% N) than Bowbeat Hill (0.77% N) despite the higher total N deposition at Bowbeat (25 kg N ha-1 y^{-1}) compared with Auchencorth Moss (14 kg N ha^{-1} y^{-1}). Although the N deposition is lower at Auchencorth, the N is derived largely from NH_3 concentrations giving an increased fraction of dry deposition to wet deposition. This is consistent with dry deposition having a larger effect on total % N of bryophytes than wet deposition. Leith *et al.* (2001) found that the response per unit of N was greater for NH_3 than for wet NH_4Cl.

By contrast, the mean soluble NH_4-N concentrations for *P. schreberi* were slightly smaller at Auchencorth Moss (1.44 µg g^{-1} FW) compared to Bowbeat Hill (1.99 µg g^{-1} FW) but the difference is not significant (p=0.123).

6.6. Whim Moss

6.6.1. Moss sampling at Whim Moss

The species of pleurocarpous moss collected for tissue N content and soluble NH_4-N concentration determination at Whim Moss was *Hypnum jutlandicum*. This species was selected, as it was one of the most frequently occurring moss species along with *Pleurozium schreberi* found growing at the Whim Moss site.

6.6.2. Sample collection and preparation for chemical analysis

The sampling methods and sample preparations were identical to those described in the methods section 6.2.1.

6.6.3. Collection of *H. jutlandicum* at Whim Moss

Samples of *H. jutlandicum* were collected at 8, 16, 32 and 60 m along the NH_3 transect and at background ambient. The mean two-year NH_3 concentrations along the transect were 70, 47, 17, 5 and 0.5 μg m^{-3} respectively. This corresponded to annual total N inputs of 22, 166, 66, 26 and 10 kg N ha^{-1} y^{-1}. Single samples of *H. jutlandicum* were collected from each of the 15 wet N deposition treatments plots (See section 3 for plot detail). In additional, samples of *H. jutlandicum* were also collected from 3 replicate plots of both the NH_4Cl and $NaNO_3^-$ (16 kg N ha^{-1} y^{-1}) treatments. This data will give additional information to the current study and will also be used as part of the Whim Moss long-term study database. Samples were collected and analysed following the methods described in Section 6.2.1.

6.6.4. Results of foliar tissue N of mosses from Whim Moss

The N content data and the soluble NH_4-N concentration data are for naturally growing *H. jutlandicum* plants, which have been exposed to two years of wet and dry N treatments at Whim Moss manipulation facility.

The mosses at Piddles Wood and Happendon Wood are dominated by dry N inputs, whereas inputs to the *Hypnum jutlandicum* at Whim Moss are dominated by wet N inputs as applied as either NH_4^+ or NO_3^-.

6.6.5. NH_3 transect

Along the atmospheric NH_3 transect, there was a strong linear relationship between tissue N content (R^2 =0.883), soluble NH_4-N concentration (R^2 =0.889) in *H. jutlandicum* and exposure to of NH_3 (expressed as log NH_3 concentration) (Figure 6.16). The NH_3 concentration used in Figures 6.16a & 6.16b is the 2-year monthly mean concentration (May 2002 to May 2004).

A similar strong linear relationship occurs between both total N content and NH_4-N with log N deposition for the NH_3 transect (Figure 6.16b). The closest relationship is between N deposition and foliar NH_4-N concentration, for which the correlation was 0.96. This is consistent with the response being to N deposition rather than NH_3 (explaining the non-linear portion of the foliar NH_4-N response to NH_3 concentration at low NH_3 concentrations, due non-NH_3 nitrogen inputs), plus the larger response of foliar NH_4-N compared with total tissue N (factor of 9 compared with factor of 2 change across the transect).

6.6.6. Wet N deposition treatments

A strong linear relationship for tissue N content is found for *H. jutlandicum* exposed to enhanced wet N treatments (Figure 6.16c).

For the wet treatments it is of interest that the response to increased N supply was different for tissue N content and soluble foliar NH_4^+. For total foliar N the response is approximately linear with the log of deposited NO_3^- and NH_4^+, with a larger response seen for ammonium than for nitrate. By contrast, the response of soluble foliar NH_4-N to wet N inputs was not linear: there was only a minor response in the range 0-32 kg N ha-1 y^{-1} and then a very large increase in response to the 64 kg N ha-1 y^{-1} treatment. The increase for the highest N

treatment was seen for both NO_3^- and NH_4^+ inputs, with the largest response for NH_4^+ rather than NO_3^- inputs.

These patterns show that both wet deposited NO_3^- and NH_4^+ affect total foliar N and soluble NH_4-N concentration, but for both parameters the response is largest for NH_4^+ (consistent with the data for the standardised grass biomonitors, Section 5). This is also consistent with annual foliar N content measurements for Whim Moss, which show that increases in foliar N per unit N are generally greater for NH_4-N than for NO_3-N. It is of particular interest to note that wet deposited NO_3^-increases foliar soluble NH_4-N concentration demonstrating the importance of nitrogen cycling in the plant between oxidized and reduced forms. The different shape of the response to wet N inputs is also of interest, with a gradual response for tissue N content, but evidence of a critical threshold (between 32 and 64 kg N ha^{-1} y^{-1} for 2 years of wet deposition treatment) for the foliar NH_4^+ values.

A relatively strong relationship between total N content and soluble NH_4-N concentration in *Hypnum jutlandicum* was found for both forms of N (Figure 6.17) after 2 years of N treatments (8, 16, 32 and 64 kg N ha^{-1} y^{-1}).

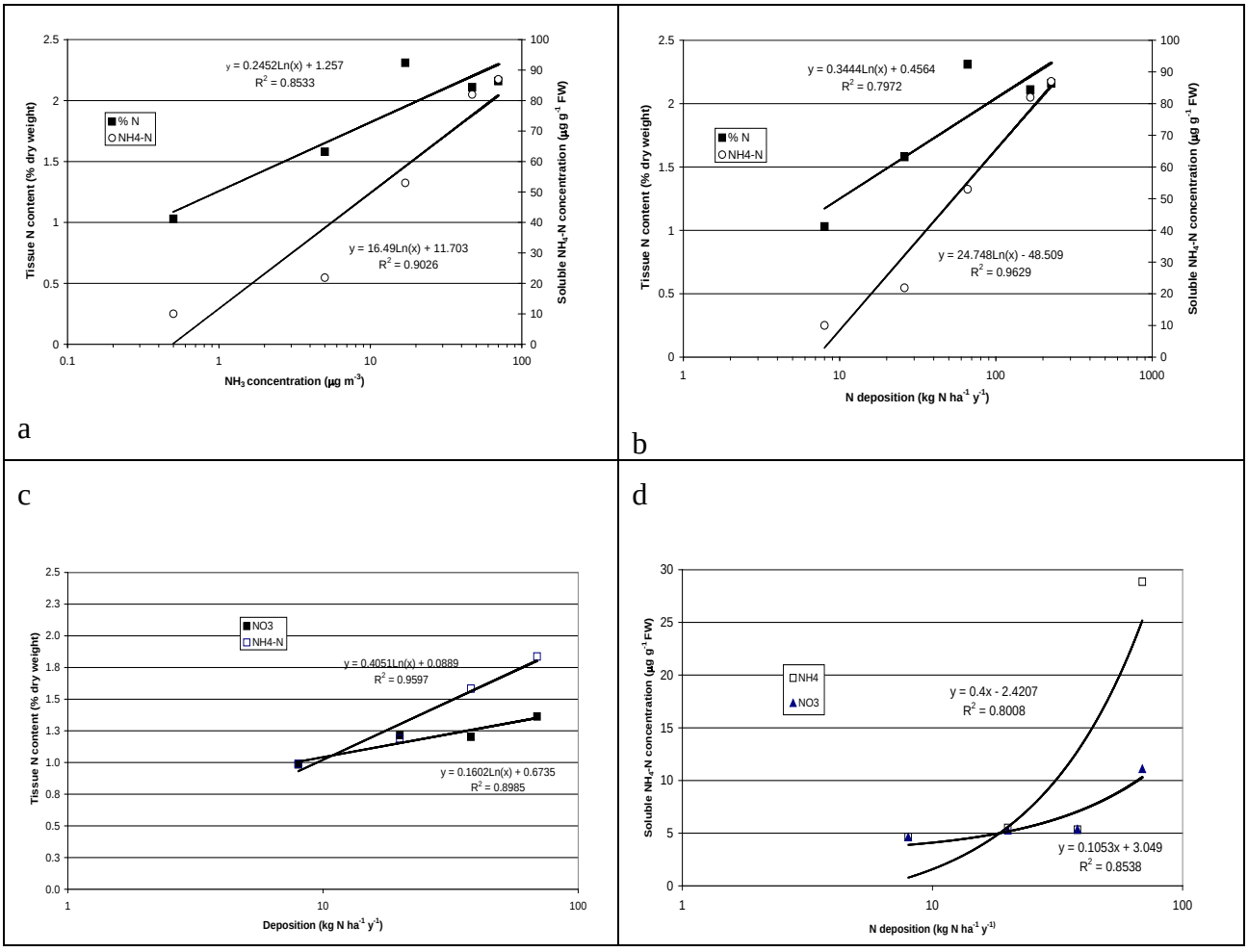

Figure 6.16. The tissue N content and the soluble NH_4-N concentration for *H. jutlandicum* along the dry N deposition NH_3 transect at Whim Moss plotted against a) NH_3 concentration and b) N deposition. Graphs 18 c and d are the N content and the soluble NH_4-N concentration for *H. jutlandicum* for the wet N deposition treatments.

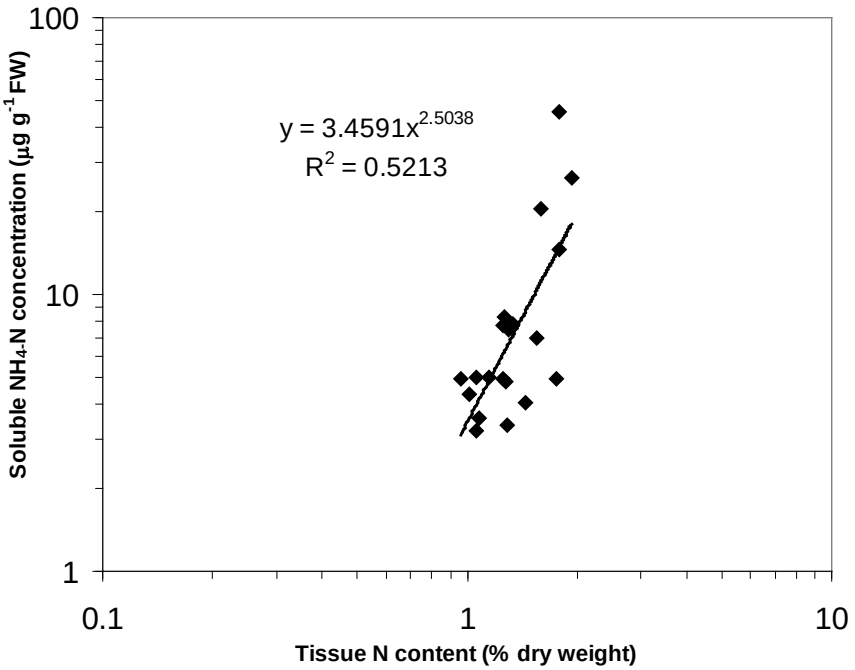

Figure 6.17. The relationship between the tissue N content and soluble NH_4-N (µg g^{-1} FW) concentration for the pleurocarpous moss *Hypnum jutlandicum* grown at Whim Moss and exposed to controlled levels of wet N deposition.

6.7. Discussion

A strong relationship between total N content and NH_4-N was found for the pleurocarpous mosses at Piddles Wood, dominated by NH_3 exposure compared to Happendon Wood, dominated by NO_2 exposure. This probably results from the large NH_3 concentration and N deposition input range at Piddles Wood (1.46 -100 µg m^{-3} and 26-1000 kg N ha^{-1} y^{-1}) compared to Happendon Wood (0.48 -1.27 µg m^{-3} and 12-23 kg N ha^{-1} y^{-1}). The smaller the atmospheric input the greater the potential variation in the foliar N measurements. The results indicate that for a site with defined large N source such as poultry unit, either N content or soluble N concentration are an effective bioindicator of current impact to a designated site and would both contribute to longer term assessment of site integrity. The relationship between tissue N content and soluble NH_4 –N concentration for wet N deposition suggests either method could be used for biomonitoring at sites with diffuse sources of N in the range 8-64 kg N ha^{-1} y^{-1}.

This curved relationship was reported in Sutton *et al*. (2004a) for tissue N content of pleurocarpous mosses exposed to high NH_3 concentrations from an agricultural source and suggests an N saturation of the moss tissue.

The difference in the soluble NH_4-N concentrations in the current study and those reported by Sutton *et al.* (2004a) for moss species sampled along a NH_3 gradient from Earlston poultry farm could be due to a number of factors. It is suspected that the use of new analytical protocols in this current study may account for some of the observed differences. The poultry farm in Sutton *et al.* (2004a) had been established for 20 plus years, whereas the Piddles Wood unit was only 10 years old. The variation in density and type of ground cover may also be important with potential shading/protection from NH_3 deposition by a dense canopy/ground cover. The moisture content of mosses when they are collected could also influence % N content.

The reasonable relationship with both N content and soluble NH_4-N concentration and distance from the M74 but not for NH_3 concentration would suggest that the NH_3 concentration was too low (range 1.27 to 0.45 μg m^{-3} along the transect) to impact on N uptake, with NO_2 being a more important influence.

The M74 has an average daily average flow of vehicle of 35,000 per day with a maximum flow of 85,000 (DfT 2000). The NO_2 concentrations 10 m from the M74 (~20 μg m^{-3}) are smaller than those reported by Bignal *et al*. (2004) for the M62 (40 μg m^{-3} at 10 m from the motorway) with a mean vehicle flow of 74,000 vehicles per day with a maximum flow of 130,000. There appears to be a good relationship between vehicle flow and NO_2 concentrations using the data from both studies with NO_2 concentrations and vehicle flow at Happendon Wood being ~ 50% lower than for the M62 data. It is considered even at the concentrations measured the atmospheric NO_2 exposure could be impacting on the mosses grown at Happendon Wood.

There could be other factors inhibiting N uptake and impacting on the mosses at sites closest to the M74 such as the cocktail of other pollutants from vehicular emissions.

The strong relationship with both total N content, soluble NH_4-N concentration and NH_3 concentration and N deposition found for the woodland moss species at Piddles Wood is consistent with the findings of the field study carried out at a poultry unit in the Scottish borders (Pitcairn *et al*. 1998, 2003; Sutton *et al.* 2004a) even though % N and soluble NH_4-N concentrations at Piddles Wood levels were lower. Exposing moorland species to enhanced NH_3 concentrations at Whim Moss gives a unique opportunity to determine the effect on mire habitats, under field conditions, of enhanced NH_3 concentrations similar to those found close to a 100,000 bird poultry unit. The results of this current study show that NH_3 concentrations are resulting in increased N foliar concentrations in *H. jutlandicum,* which could lead to changes in integrity of this species and probably other N sensitive mire species.

The determination of wet dominated N deposition impacts from diffuse sources on semi-natural habitats is difficult, but is important to the conservation agencies for condition assessment monitoring of habitats. The results from the current study at Whim Moss show that for *H. jutlandicum* the form of N exposure influences the overall N uptake, with NH_4^+ treatments increasing to a greater extent than NO_3^- treatments per unit N applied. The tissue N response to enhanced wet N deposition was also found to be greater than that for soluble NH_4-N concentrations. This response to N form has not been seen as strongly in other moss species exposed to N treatments at Whim Moss. This suggests that there could be inter-specific variation in moss species to the form of N (NH_4^+ or NO_3^-) in precipitation. The influence of N form on N uptake is being currently studied at Whim Moss for a range of blanket bog species and is an important question for stakeholders. The study also shows that after 2 years of wet N treatments there is only an effect on tissue N content and to a lesser extent soluble NH_4-N concentration at N deposition between 16 and 32 kg N ha^{-1} y^{-1}. Although, there is a strong linear increase in total N concentration with log N deposition in *H. jutlandicum* after 2 years exposure there is no indication yet of decline in this species due to enhanced N deposition.

6.8. Conclusions

- There were strong, robust relationships between tissue N content and soluble NH_4-N concentration within the moss tissue and atmospheric NH_3 concentration and N deposition at Piddles Wood.
- Foliar N concentration methods could be use effectively as bioindicators of atmospheric N impacts at designated sites with a strong local N point source.
- Differences in species sensitivity were found between *E. striatum* and *E. praelongum* with the latter appearing to be N saturated at high atmospheric NH_3 concentrations and total N deposition.
- There was a good relationship between N content and soluble NH_4-N concentration and distance at the M74 motorway site at Happendon Wood. The foliar N concentrations decreased with increased distance away from the pollutant source (i.e. M74 motorway). As the atmospheric NH_3 concentrations measured were consistently low along the transect this suggests that other factors (including NO_2 concentrations) could influenced N uptake by the moss.
- There was a poor relationship between tissue N content (% dry weight) and NH_3 concentration and N deposition at the M74 motorway site (Happendon Wood). However, there were strong relationship between and soluble NH_4-N concentration and NH_3 concentration and N deposition, indicating differences in sensitivity between the two foliar N methods.
- There was also a strong relationship between both tissue N content (% dry weight) and soluble NH_4-N concentration with changes in atmospheric NO_2 concentration and NO_2 deposition, suggesting that N derived from NO_2 pollution is impacting on the mosses at Happendon Wood.
- The total N content of *P. schreberi* was significantly higher at the lower wet N deposition site at Auchencorth Moss than Bowbeat hill.
- In contrast, the mean soluble NH_4-N concentrations for *P. schreberi* were slightly smaller at Auchencorth Moss than Bowbeat Hill but were not significant.
- The results for the diffuse wet N deposition sites suggest that N is not impacting at these sites and the use of the foliar N bioindicator methods would only be applicable with long-term monitoring as an 'early warning' indicator for increases in N deposition effects.
- There were strong log-linear relationships between increasing tissue N content (% dry weight) and soluble NH_4-N concentration with increasing NH_3 concentration (ranging from 0.5 $\mu g\ m^{-3}$ to 70 $\mu g\ m^{-3}$) along the NH_3 transect at Whim Moss.
- Differences were found between N forms in the wet N treatments. There was a strong linear-log relationship between tissue N content (% dry weight) and soluble NH_4-N concentration and wet N deposition of both N forms. However, the increases in foliar N per unit N were greater in the NH_4^+ treatments than the NO_3^- treatments.

- The tissue N content (% dry weight) increased significantly when compared to ambient N deposition (10 kg N ha^{-1} y^{-1}) in both forms of N at N deposition treatments, 32 and 64 kg N ha^{-1} y^{-1}.
- In contrast soluble NH_4-N concentrations were only significantly increased in the NH_4Cl, 64 kg N ha^{-1} y^{-1} treatment. This result suggesting a critical threshold for wet deposition may have been exceeded in this treatment for soluble NH_4^+ concentrations.

7. Lichen diversity: intensive sites

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7.1. Introduction

The application of epiphytic lichen indicator species to detect increasing atmospheric ammonia associated with intensive stock farming was developed in the Netherlands by analysing data from lichen communities on trunks of oak trees to determine nitrophyte and acidophyte indicator species (van Herk 1999, 2002). This method was applied and assessed at two sites in the UK under different climatic conditions; in the more continental climate of Norfolk and the oceanic climate of south west Devon, where ammonia monitoring data were available. In addition a sampling protocol devised for lichen communities of twigs was used (Wolseley and Pryor 1999) and combined with van Herk (1999, 2002) indicator species. The results suggested that lichen communities on twigs may be a better indicator of current atmospheric conditions than lichens on trunks (Wolseley and James 2000). The development of a standard European method for sampling trunks (Asta *et al*. 2000) allowed an improvement in the measurement of lichen frequency on trunks and this combined with twig sampling and the use of Ellenberg lichen values (Wirth 1992) was applied along a transect from an ammonia source in Berwickshire Scotland (Sutton *et al*. 2004a; Sutton *et al*. 2004b). The data from these surveys suggested that; a) nitrophyte values were increasing on twigs before trunks in sites of increasing ammonia concentrations, b) loss of acidophyte species occurred at lower levels of ammonia concentrations than the increase in nitrophyte species and c) the climate gradient in Britain had a strong affect on both nitrophyte and acidophyte communities. Finally, the species in the indicator lists developed for the Netherlands were not always appropriate for use as indicators in the oceanic climates of UK. This also applied to Ellenberg values, which were assessed by Wirth (1992) for application in central Europe.

The increase in deposited nitrogen is widespread in Britain and is associated with rural sites where the intensification of farming methods has occurred and also areas of high rainfall in the west where long range N is deposited. The selection of intensive sites within this study through a range of atmospheric and climatic conditions across Britain allows us to compare the application of lichens as bioindicators of N deposition within different pollution contexts. By contrast, an extensive survey using macrolichens permits the results from the detailed surveys to be extended in a simple way for application by conservation agency staff.

7.2. Methods

Sites were selected for intensive study in Piddles Wood, Dorset adjacent to an intensive poultry unit, Happendon Wood adjacent to the M74 in Lanarkshire, Whim Moss in the Scottish Borders where controlled wet deposition of NH_3 is established and Bowbeat Hill in the Scottish Borders, Scotland at c. 800 m. For a full description of individual sites see Section 3.

For each of the intensive survey sites, samples were recorded along a transect from the point source within the close vicinity of atmospheric ammonia sampling tubes.

Where possible, trees of the same species with naturally low pH bark were selected up to 20 m from the atmospheric monitoring and sampling site. Where tree species varied across the

transect recording was undertaken on dominant and restricted low pH barked trees, in order to correlate the effect of tree species and bark pH on epiphytic lichen communities across the transect.

7.2.1. Lichen diversity

Between 3-5 exposed trees with girths >40cms were selected at each sampling point and the standard LDV laddered quadrat of five 10 x 10 cms quadrats pinned on 4 compass aspects. Frequency of each species was recorded on each aspect and frequencies summed for each tree as for extensive survey (an example of the sampling forms are shown in Appendix III).

Bark with as smooth as possible surface (~1 cm diameter sample) was collected from all trunks and up to 3 samples per trunk air dried in paper bags for estimating surface bark pH in the lab.

Twigs were sampled, if possible on the same tree species and on a well-exposed aspect, while shaded aspects were avoided. Up to 10 twigs were sampled at each transect point. Girdle scars were counted along the twig in order to divide samples into 3 age classes, 1-5 years, 6-10 and 11+. It was not always possible to estimate age after 8 years so that the 3rd section was often measured as a further metre.

Lichens were recorded on twigs with a frequency of 1-3 for each twig. Where a species occurred on all 3 age classes, the number were doubled (e.g. 3 records were scored as 6). For explanation of this see van Herk (1999, 2002). More or less straight twig samples ~5-7 mm in diameter and 10-15 cm long were collected and air dried in paper packets in order to measure pH in the lab.

7.2.2. Bark pH

Samples were stored for lab sampling of both trunk and twig pH. The method used on tree bark followed Farmer *et al*. (1990). The surface was wetted with 25 mM KCl for 5-10 minutes and the pH measured in the lab with a Gelplas surface electrode.

The method used to measure bark pH of twigs was modified from Kermit and Gauslaa (2001). In the lab 6 cm lengths of twigs 5-7 mm thick were cut and the cut ends sealed with paraffin wax, then placed in a tube containing 6 ml of 25 mM KCl. Samples were shaken and incubated for 1-2 hours at room temperature. Samples were shaken before removing small amounts with a pipette and recording pH of the liquid with the same electrode.

7.2.3. Data entry

Data were entered in Access files and a range of scoring methods used.

Nitrophyte and acidophyte indicators.

Species on trunks were classified as nitrophyte or acidophyte species according to van Herk (1999, 2002) and as used by Wolseley and James (2002b). Total scores were averaged from sampled trees for each site for nitophytes and acidophytes (maximum score 20) and for diversity. On the UK extensive survey only macrolichens were recorded, whereas on the intensive survey macro and crustose lichens were recorded. Species on twigs were classified as nitrophytes or acidophytes according to van Herk (1999, 2002) and as used by Wolseley and James (2002b). If a species occurred on all three segments of the twig the score was doubled to six. The total score was then averaged for each site for nitrophytes and acidophytes and for species diversity.

Ellenberg scale. Lichens were scored on each trunk and twig for environmental factors according to Wirth (1992) including nutrient status, toxitolerance and moisture (Wirth, 1992). The Ellenberg scales of 1-9 relate to each species' tolerance of the factor concerned, so that high scores indicated a tolerance of nitrogen. Species recorded on the present surveys in all sites are shown in Table 7.2 with Ellenberg values for 3 environmental factors. No cover weighting was created for lichens.

Wirth (1992) noted that these lists showed good correlations with environmental data for central Europe, but would need evaluating for other geographical regions. This has not been done for Britain where climatic conditions vary from continental to oceanic.

7.3. Results

Sites were not all suitable for epiphytic sampling due to the following factors:

- Tree species were either not available or included a range of different species e.g. Whim Moss and Bowbeat Hill.
- Transects crossed a range of substrate and geographical conditions exhibiting marked changes in exposure and humidity e.g. Happendon Wood.

Application of nitrophyte, acidophyte and Ellenberg values.

All species were assigned nitrophyte and acidophyte categories according to van Herk (1999, 2002 (Table 7.1) and Ellenberg values for nutrients, toxitolerance and moisture (Wirth, 1992) (Table 7.2).

Table 7.1. Nitrophyte and acidophytes species identified in the intensive sites (after van Herk 2002). Macrolichens indicated with *.

Nitrophytes	Acidophytes
Candelariella reflexa	*Cladonia* spp.*
*Hyperphyscia adglutinata**	*Evernia prunastri**
*Phaeophyscia orbicularis**	*Hypocoenomyce scalaris**
*Physcia adscendens**	*Hypogymnia physodes**
*Physcia tenella**	*Hypogymnia tubulosa**
*Xanthoria candelaria**	*Lecanora conizaeoides*
*X. parietina**	*L. pulicaris*
*X. polycarpa**	*Lepraria* spp.
	*Parmelia saxatilis**
	*Platismatia glauca**
	*Pseudevernia furfuracea**
	Usnea sll species*

Table 7.2. Ellenberg score values given for all species in the extensive surveys (Wirth 1992).

Species	Nutrient	Toxi-tolerance	Moisture
Amandinea punctata	5	9	3
Arthonia spadicea	3	5	4
Arthopyrenia sp.	3	4	4
Buellia pulverea	5	9	
Bryoria fuscescens	2	4	6
Candelaria concolor	4	5	3
Chaenotheca furfuracea	2	3	4
Chrysothrix candelaris	2	4	4
Cladonia polydactyla	1		7
Diploicia canescens	6	8	5
Dimerella pineti	6	3	4
Evernia prunastri	3	6	3
Flavoparmelia caperata	3	3	4
Hypocoenomyce scalaris	2	8	3
Hypogymnia physodes	2	8	3
Hypogymnia spp.	3	5	3
Hypogymnia tubulosa	3	6	3
Hypotrachyna revoluta	3	4	6
Lecania cyrtella	6		2
Lecanora chlarotera	4	6	3
Lecanora conizaeoides	x	9	
Lecanora expallens	4	9	3
Lecanora intumescens	3	3	6
Lecanora symmicta	4	4	5
Lecidella elaeochroma	4	6	3
Lepraria sp.	3	9	3
Leproloma vouauxii	4	1	5
Melanelia exasperata	5	1	6
Melanelia exasperatula	4	6	3
Melanelia glabratula	3	6	4
Melanelia laciniatula	4	4	6
Melanelia subaurifera	4	4	3
Micarea prasina	3	4	4
Mycoblastus sanguinaria	1		7
Mycoblastus sterilis	3	5	4
Ochrolechia androgyna	2	4	7
Parmelia saxatilis	2	7	5
Parmelia subrudecta	3	6	3
Parmelia sulcata	4	8	3
Parmelina pastilifera	4	3	7
Pertusaria albescens	4	4	3
Pertusaria amara	2	5	4
Pertusaria hymenea	3	3	5
Pertusaria leioplaca	3	5	4
Pertusaria pertusa	3	4	5
Phaeographis smithii	5	3	?
Phlyctis argena	3	6	4
Physcia adscendens	6	8	3
Physcia aipolia	5	4	3
Physcia leptalea	5	4	3
Physcia tenella	6	8	3
Platismatia glauca	2	5	5
Pseudevernia furfuracea	2	1	3
Punctelia subrudecta	3	6	3
Pyrenula macrospora	3	5	4
Ramalina farinacea	3	6	4
Ramalina fastigiata	5	2	6
Rinodina sophodes	5	1	7
Scoliciosporum	5	8	3
Sphaerophorus globosus	1	1	9
Thelotroma lepadinum	2	2	7
Usnea	3	2	6
Usnea ceratina	3	2	7
Usnea cornuta	3	2	6
Usnea florida	3	2	7
Usnea subfloridana	3	3	6
Xanthoria candelaria	7	5	3
Xanthoria parietina	6	7	3

7.3.1. Piddles Wood

Piddles Wood is a Site of Special Scientific Interest adjacent to a poultry unit and consisting of oak woodland with ash including coppice and veteran trees and an area of secondary scrub.

Five ammonia sampling sites were established at intervals of 5, 20, 40,100 and c. 250 m from the poultry unit. Oak trees *Quercus* spp. were the preferred substrate for sampling, but at site 2 there was a patch of damp woodland dominated by *Fraxinus* (ash) with only one oak tree in the area between sites 2 and 3. No twigs of ash were accessible at site 2 and therefore twigs of *Acer campestre* were sampled. Sites 1-3 were rather densely shaded while site 4 was in a glade where several large oaks stood, and site 5 was on the edge of young secondary scrub. Trees > 1 m girth were used in the vicinity of the ammonia monitoring site where possible and younger ones if not.

Lichen diversity
74 species were recorded in the sampling sites in Piddles Wood including 20 macrolichen species and 34 crustose species (Table 7.3). On large well-lit oak trees in sampling site 4 (100m from the source), the old forest indicator crustose species *Lecanactis abietina* was present but not recorded in the quadrats.

Nitrophyte and Acidophyte values
Acidophyte and nitrophyte values were calculated on trunks and twigs of *Quercus* where possible (Table 7.4). Nitrophyte values for trunks were highest at site 3, 20 m from the source and show a rapid drop to site 4, 100 m from the N source. Acidophyte values for trunks are high due to the occurrence of a single species *Lepraria incana,* which persists and is abundant at site 3 at 40 m from the source of NH_3. In contrast there are no acidophytes on the twigs before site 5 at 250 m from the source, reflecting a much greater sensitivity of twig acidophytes to NH_3 (Figure 7.1).

Table 7.3. Species recorded on sampled trees in Piddles Wood showing presence on trunks and twigs, macrolichen and crustose habits, acidophyte (A) or nitrophyte (N) according to van Herk (2002).

Piddles Wood	Trunk	Twig	Macr/ Crust	N or A
Amandinea punctata		X	C	
Anisomeridium biforme	X		C	
Arthonia didyma	X		C	
Arthonia sp.		X	C	
Arthonia spadicea	X		C	
Arthopyrenia sp.		X	C	
Buellia pulverea		X	C	
Chrysothrix candelaria	X	X	C	
Cladonia chlorophaea	X		M	A
Cladonia coniocraea	X		M	A
Cladonia macilenta	X		M	A
Cladonia polydactyla	X		M	A
Cladonia sp.	X		M	A
Cliostomum griffithii	X		C	
Diploicia canescens	X		M	N
Evernia prunastri	X	X	M	A
Flavoparmelia caperata	X	X	M	
Fuscidea lightfootii	X	X	C	
Graphhis sp.		X	C	
Graphis elegans	X		C	
Hypogymnia physodes		X	M	A
Hypogymnia sp	X	X		A
Hypogymnia tubulosa		X	M	A
Hypotrachyna laevigata	X	X	F	
Hypotrachyna revoluta	X	X	M	A
Lecanora albella		X	C	
Lecanora chlarotera	X	X	C	
Lecanora confusa		X	C	
Lecanora conizaeoides	X	X	C	
Lecanora expallens	X	X	C	
Lecanora jamesii	X		C	
Lecidella elaeochroma		X	C	
Lepraria	X	X	C	A
Leproloma vouauxii	X		C	
Melanelia exasperata		X	M	
Melanelia exasperatula	X		M	
Melanelia glabratula	X	X	M	
Melanelia laciniatula		X	M	
Melanelia sp.	X	X	M	
Melanelia subaurifera	X	X	M	
Micarea prasina	X		C	
Mycoblastus sterilis	X		C	
Parmelia saxatilis	X		M	A
Parmelia sp.		X	M	
Parmelia sulcata	X	X	M	
Parmelina pastilifera		X	M	
Parmotrema chinense	X	X	M	
Pertusaria albescens	X		C	
Pertusaria amara	X		C	
Pertusaria hymenea	X		C	
Pertusaria leioplaca		X	C	
Pertusaria pertusa	X		C	
Phaeographis smithii		X	C	
Phlyctis argena	X		C	
Physcia adscendens		X	M	N
Physcia aipolia		X	M	
Physcia leptalea	X		M	
Physcia tenella	X	X	M	N
Platismatia glauca	X	X	M	A
Pseudevernia furfuracea		X	M	A
Punctelia borreri		X	M	
Punctelia subrudecta	X	X	M	
Pyrenula macrospora		X	C	
Ramalina calicaris		X	M	
Ramalina farinacea	X	X	M	
Ramalina fastigiata	X	X	M	
Rinodina sophodes		X	C	
Scoliciosporum chlorococcum	X	X	C	
Sphaerophorus globosus	X		M	
Thelotremia lepadinum	X	X	C	
Usnea sp.	X		M	A
Usnea ceratina		X	M	A
Usnea cornuta	X	X	M	A
Usnea florida	X	X	M	A
Usnea subfloridana	X	X	M	A
Xanthoria candelaria	X	X	M	N
Xanthoria parientina	X	X	M	
Xanthoria polycarpa	X	X	M	
Xanthoria sp.	X	X	M	

Table 7.4. Bark pH of *Quercus*, ammonia concentration (μg m^{-3}) and acidophyte (AV) and nitrophyte (NV) values for trunks and twigs at Piddles Wood, Dorset.

Piddles wood	Site 1	Site 2	Site 3	Site 4	Site 5
Distance (m)	5	20	40	100	250
NH_3 conc.	101	31.35	17.08	1.95	1.46
pH trunk Qu	4.6		5	5.2	4.8
pH twig Qu	6.4		5.8	5.2	5.1
Trunk AV	0(Qu)	3.5 (Fx,Qu)	11(Qu)	7.6(Qu)	3(Qu)
Trunk NV	2(Qu)	11 (Fx,Qu)	4.8(Qu)	0.2(Qu)	0(Qu)
Twig AV Qu	0		0	0	0.67
Twig NV Qu	1.8		5	8.75	3

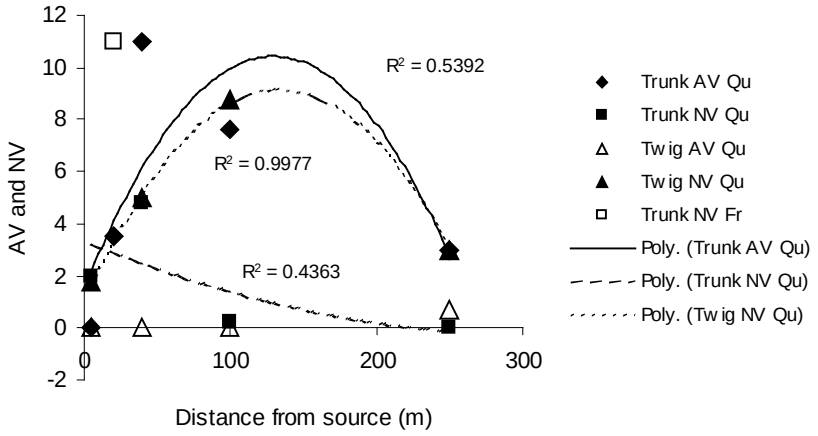

Figure 7.1. Nitrophyte values (NV) and acidophyte values (AV) for *Quercus* (Qu) trunks and twigs and high NV for *Fraxinus* (Fr) with distance from source at Piddles Wood showing polynomial regression of AV-NV for trunks and twigs (poly). (Acidophytes only occurred on twigs at site 4 250 m from the poultry unit, value 0.67, whereas *Lepraria incana* (scored as an acidophyte) is dominant on trunks in station 2-4. If this were excluded acidophytes only appeared on trunks in station 4).

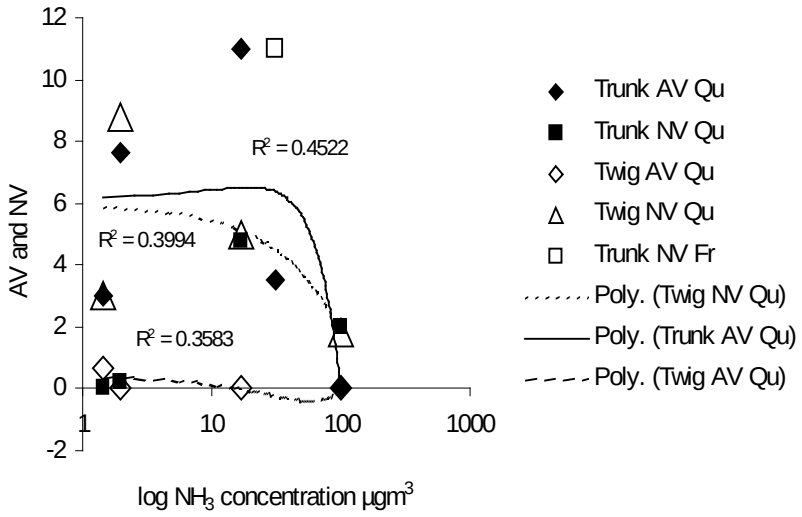

Figure 7.2. Nitrophyte values (NV) and acidophyte values (AV) for *Quercus* (Qu) trunks and twigs and high NV for *Fraxinus* (Fr) against log ammonia concentration, showing drop in NV values at ammonia concentrations >50 µg m^3 for trunks and at lower concentrations on twigs. R^2 given for polynomial regression. Note that AV values on trunk are high due to inclusion of *Lepraria incana* as an acidophyte

Bark pH and NH_3 show a similar pattern, in that the bark pH of trunks remains low at high levels of NH_3 while the bark pH of twigs is higher (Figure 7.3). It is of interest that the lowest trunk bark pH is adjacent to the NH_3 source in sampling site 1. At sites 4 and 5 at 100 and 250m from the source respectively, where NH_3 concentrations are <3 µg/m^3, the bark pH of trunks and twigs is almost the same. Thus overall, closer to the farm, with higher NH_3 concentrations there is a tendency towards increasing bark pH of twigs, while for the trunks there is an initial increase, but a decrease very close to the source. This decrease of trunk pH immediately adjacent to the farm could indicate that some of the deposited nitrogen is nitrified, from particles emitted from the poultry farm, and such an effect was also reported for a farm in southern Scotland by Sutton *et al.* (2004a).

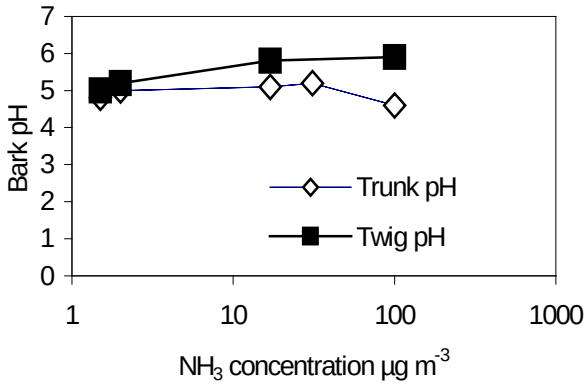

Figure 7.3. Bark pH against log ammonia concentration showing increase in bark pH of twigs with increasing ammonia, lowest bark pH of trunks were located at the sampling site adjacent to the NH_3 source.

Sutton *et al*. (2004a) showed that the subtraction of the nitrophyte score from the acidophyte score could provide a useful index of whether a sampling location was acidophyte or nitrophyte dominated. This is shown in Figure 7.4 at Piddles Wood. In this graph high AV scores due to dominance of *Lepraria incana* obscure the effects of increasing NV shown in Figure 7.2. *Fraxinus* trunks sampled at site 2 have been excluded from the NV value. On

twigs the graph shows increasing NV values with distance up to 100 m and AV recovery at 250 m.

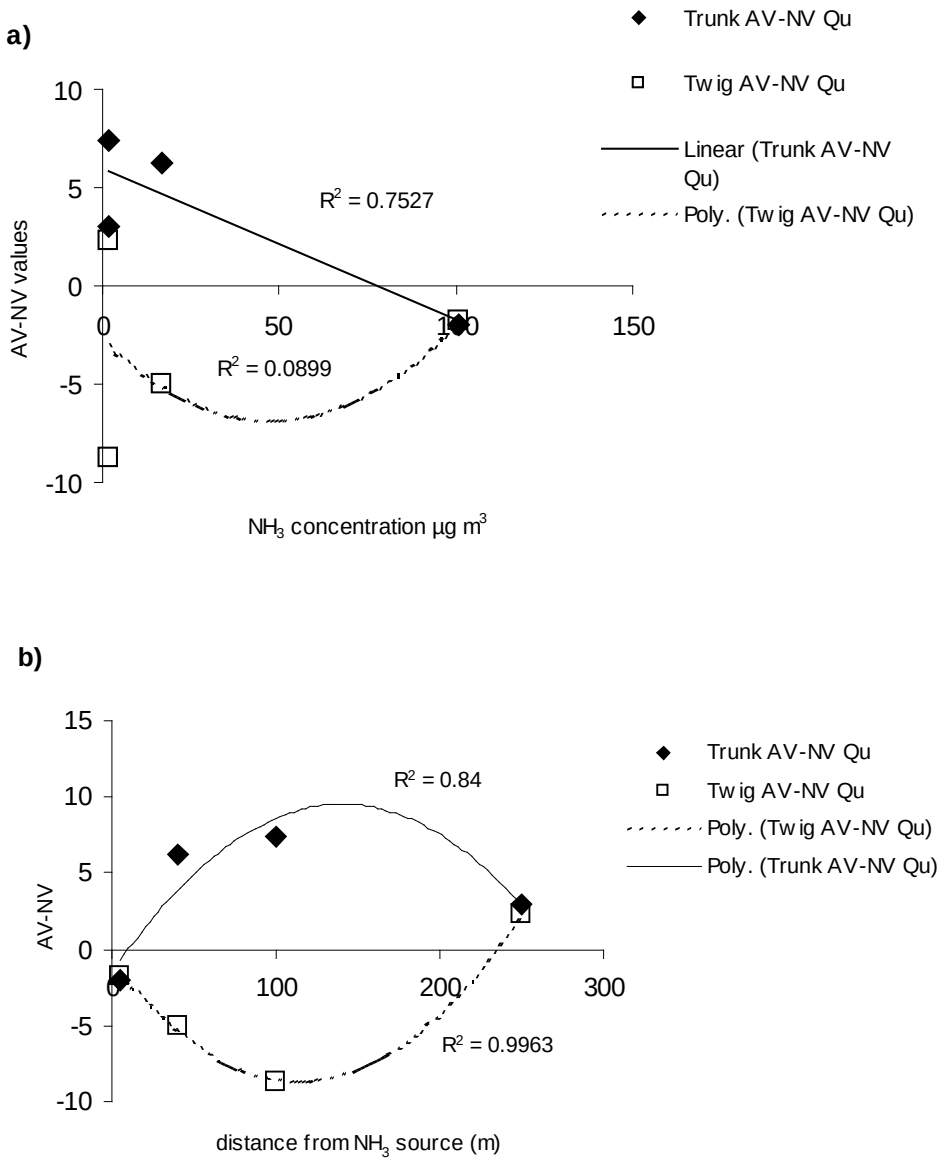

Figure 7.4. AV-NV for *Quercus* at Piddles Wood, Dorset plotted against a) ammonia concentration and b) distance from source, showing dominance of AV values in disturbed area adjacent poultry units where *Lepraria incana* was abundant, and that combined values give a better correlation with NH_3 and distance.

Ellenberg values for N and toxi-tolerance

Results are shown in Figure 7.5. Ellenberg N values were low on oak trunks at all sampling points. The highest values occurring at sampling point 4, 100m from source, due to higher numbers of recorded species resulting in higher scores, again suggesting that Ellenberg N values were also low where very high NH_3 levels occurred. High toxi-tolerant values on oak in sampling points 3 and 4 were due to the high frequency of *Lepraria incana*.

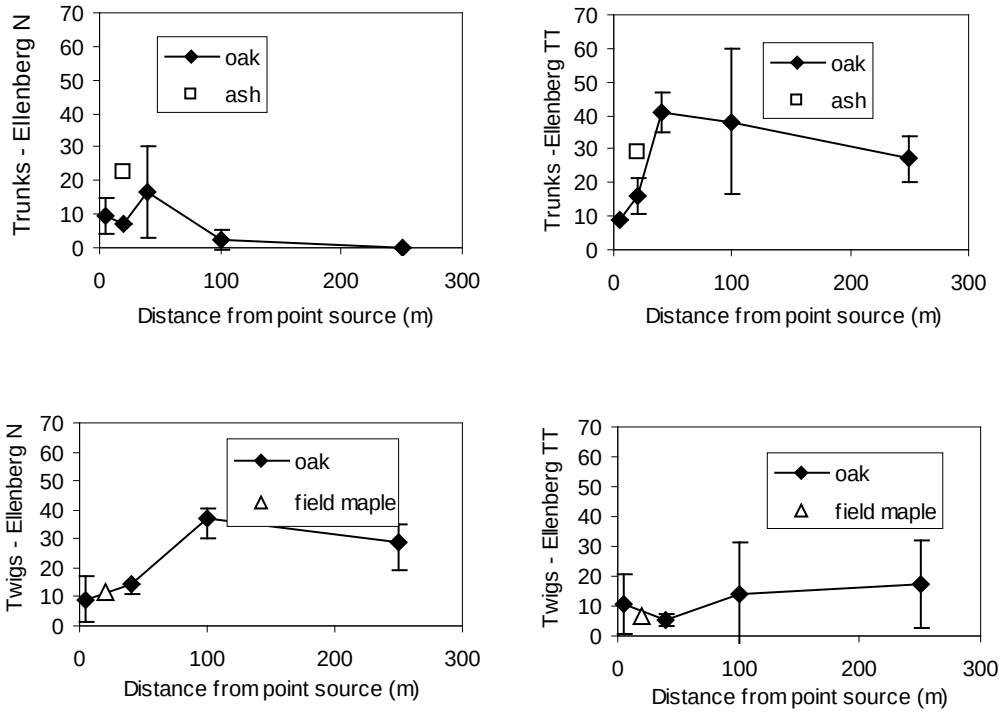

Figure 7.5. Ellenberg values for Nitrogen (N) and toxi-tolerance (TT) on *Quercus* (oak), *Fraxinus* (ash) and *Acer campestre* (field maple) twigs in sampling sites 1-5 showing low N values on trunks, with N values on trunks and twigs increasing up to 50 and 100m from source respectively and falling thereafter.

7.3.2. Happendon Wood

This site on the north east side of the M74 motorway and is also bisected by the B7078 as shown in Figure 7.6and described in Section 7. This site had a variety of tree species and ages from densely shaded young pine plantations within 10m of the motorway (sites 1 and 2) to standard trees of beech and oak in open wood pasture in site 4. Site 3 was adjacent to a sewage plant with young secondary woodland mainly *Betula*. Site 5 was in a young pine plantation in a wetland site with a few large oaks along the margins. This site presented difficulties in sampling with both tree species and accessible twigs. In order to be able to compare lichen diversity on different tree species across the transect both *Pinus sylvestris* and *Quercus* spp. were sampled where possible.

Results

Lichen diversity

Thirty seven lichen species were recorded on twigs and trunks of *Pinus sylvestris*, *Betlua* spp. and *Quercus* spp. Of which 25 were crustose species and 12 macrolichens (Table 7.5).

Table 7.5. Epiphytic species recorded on sampled trees along the transect from the motorway at Happendon Wood showing presence on trunks and twigs, foliose and crustose habits, acidophyte or nitrophyte according to van Herk (2002).

Happendon wood	Trunk	Twig	Macro/crust	N or A
Arthonia punctiformis		X	C	
Bacidia naegelii		X	C	
Bryoria fuscescens	X		M	
Chaenotheca furfuracea	X		C	
Chrysothrix flavovirens	X		C	
Cladonia sp.	X		M	
Cliostomum griffithii	X		C	
Dimerella pineti	X		C	
Evernia prunastri	X	X	M	A
Fuscidea lightfoottii		X	C	
green crust	X	X	C	
Hypocoenomyce scalaris	X		M	N
Hypogymnia physodes	X	X	M	A
Hypogymnia spp.		X	M	A
L. compallens	X	X	C	
L. conizaeoides	X		C	
L. expallens	X	X		
L. pulicaris		X	C	A
L. symmicta		X	C	
Lecanactis abietina	X		C	
Lecanora chlarotera	X	X	C	
Lepraria spp.	X	X	C	A
Melanelia subaurifera	X	X	M	
Micarea sp.		X	C	
Mycoblastus fucatus	X		C	
Ochrolechia androgyna	X		C	
P. pertusa	X		C	
P. sulcata	X	X	M	
Parmelia saxatilis	X		M	A
Pertusaria amara	X		C	
Pertusaria hymenea	X		C	
Physcis tenella		X	M	N
Platismatia glauca	X	X	M	A
Pseudevernia furfuracea		X	M	A
Pyrrhospora quernea	X		C	
Ramalina farinacea		X	M	
Rinodina sophodes		X	C	
Scoliciosporum chlorococcum		X	C	
Usnea sp.	X		M	A
Usnea subfloridana		X	M	A
Xanthoria polycarpa		X	M	N

Nitrophyte and acidophyte values

Acidophytes and nitrophyte values were calculated on trunks and twigs of *Pinus sylvestris* and *Quercus* where possible (Table 7.6). Mean values on trunks are scored out of 20 and mean values on twigs out of 3. Nitrophytes were absent on *Pinus sylvestris* and present on *Quercus* at low frequency (Figure 7.6), but on trunks increasing between sites 4 and 5 with distance from the road. Acidophytes on *Pinus* decreased with distance from the road on both trunks and twigs, whereas on *Quercus* the highest acidophyte value was at site 4 associated with a higher pH. The *Quercus* at site 5 is adjacent to an area of wetland where *Pinus* bark pH was also lowest.

This site demonstrates how many factors are influencing lichen communities and that bark pH may be affected by the substrate of the trees as well as atmospheric pollutants. The low bark pH of site 5 of both oak and pine is probably due to the acid wetland and not to atmospheric conditions. However the presence of *Lecanora conizaeoides* on pine trees adjacent to the

motorway, where AV is low due to very low diversity, suggests that there are acidifying atmospheric factors influencing the lichen flora at this site.

Table 7.6. Results of lichen survey at Happendon Wood including: bark pH of *Pinus sylvestris* and *Quercus*, ammonia concentration µg/m^3, acidophyte (AV) and nitrophyte (NV) values for trunks and twigs.

	Site 1	**Site 2**	**Site 3**	**Site 4**	**Site 5**
Distance (m)	10	20	38	150	200
NH_3 conc.	1.27	0.49	0.94	0.45	0.35
NO2 conc.	13.39	11.51	13.93	6.33	5.57
Ps-pH trunk	3.7	3.9	4	4.9	3.9
Qu-pH trunk				5.11	3.2
Ps-pH twig	4.6	4.4	4.5		4.9
Qu-pH twig				5.4	5
Trunk AV Ps	0.86	2.25	1.5	2.6	6
Trunk AV Qu				24.8	15
Trunk NV Ps	0	0	0	0	0
Trunk NV Qu				5.5	0.5
Twig AV Qu				2.6	5.67
Twig AV Ps	19	27	24.3	18	24.75
Twig NV Ps	0	0	0	0	0
Twig NV Qu				2.8	1.67

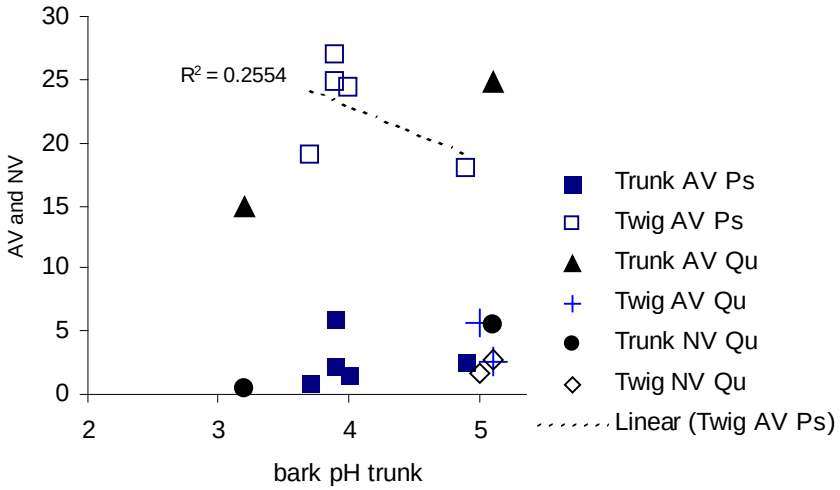

Figure 7.6. Acidophyte values (AV) and nitrophyte values (NV) on *Pinus sylvestris* (Ps) and *Quercus spp.* (Qu) at Happendon transect plotted against bark pH.

The influence of all forms of nitrogen at Happendon is shown in Figure 7.7 where the correlation of AV on twigs is better than that for trunks. All forms of N deposition contribute to loss of acidophytes species on twigs, whereas in this environment there are very few nitrophytes, therefore this is not associated with an increase in nitrophytes.

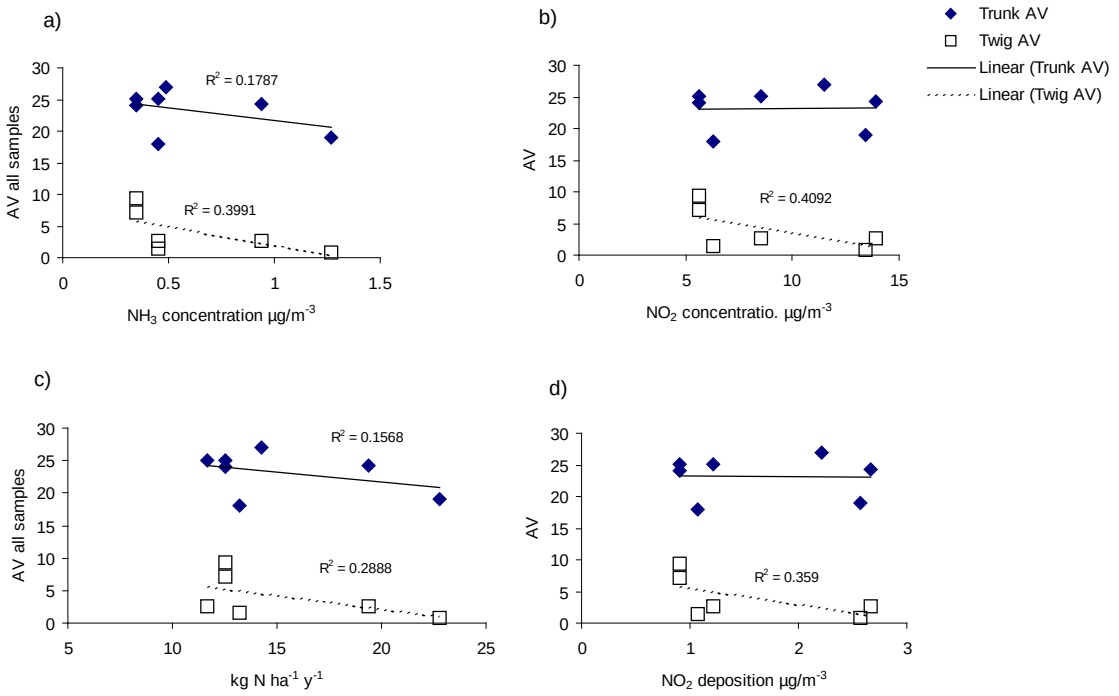

Figure 7.7. Acidophyte value of lichens on trunks and twigs of all species plotted against a) ammonia concentration b) NO_2 concentration c) total N and d) NO_2 deposition showing better negative correlation of AV values with all forms of nitrogen.

Ellenberg N values

Ellenberg values for Nutrient and for toxi-tolerance (Figure 7.8) show the discrepancy between values on oak and pine due to high diversity on oaks giving higher values (see table 7.1), and the similarity between N values and toxi-tolerance values for both species.

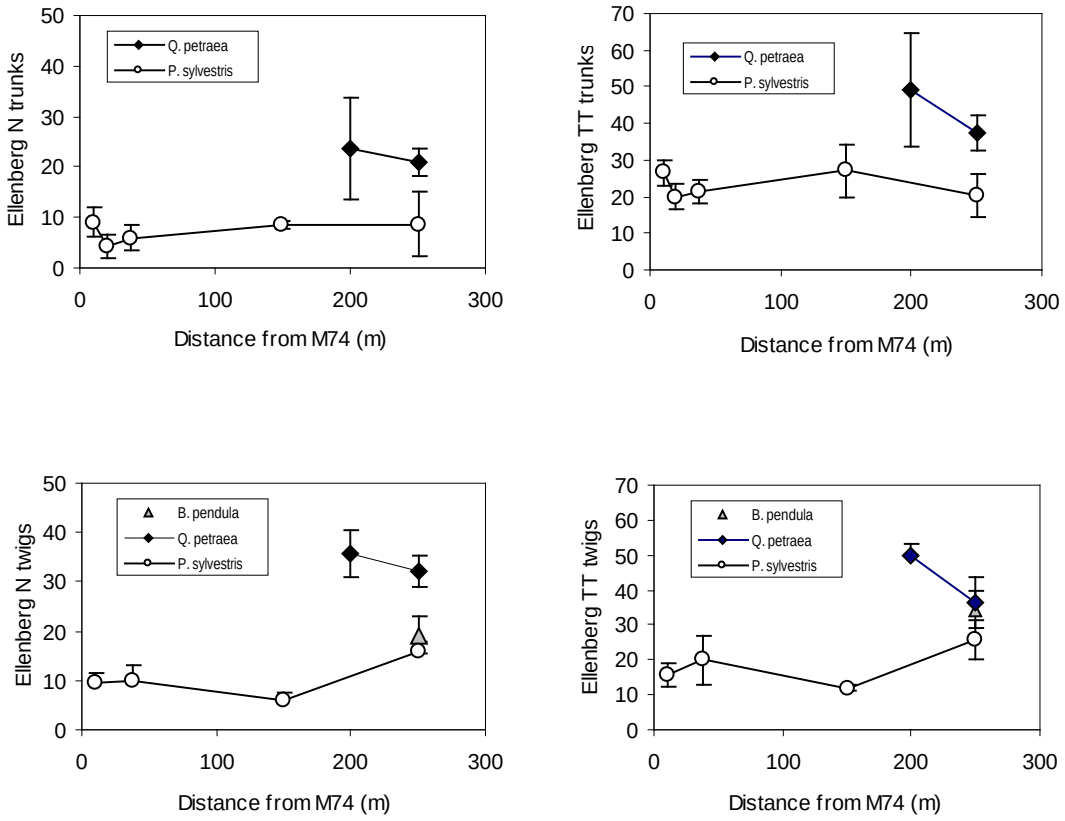

Figure 7.8. Ellenberg values for *Pinus sylvestris* (Ps) and *Quercus* (Qu) and one for *Betula* spp. (Bp) on trunks and twigs along the transect at Happendon Wood showing higher values for N and toxitolerance on oaks due to higher diversity on oaks than on pine.

7.3.3. Whim Moss

The site for the experimental deposition of NH_3 at Whim Moss is an open area of wetland formally used for peat extraction without any trees on the site itself but a scattering of young *Pinus sylvestris* that have invaded from the adjacent pine- and birch- dominated SSSI. In parts there are also young trees of *Betula pubescens*. In order to assess variation with tree species *Pinus* trees were sampled within c.10m of the broad walk and *Pinus* and *Betula* trees c. 100m to the east of the site.

Results

Epiphytic lichens recorded at Whim Moss on *Pinus sylvestris* are all acidophytes, being the characteristic flora of pine trees in Scotland with a low bark pH (Table 7.7). The only nitrophyte species present (as defined by van Herk, 2002) was *Lecanora pulicaris* occurring on birch twigs. This species is widespread in Scotland on twigs and younger trunks.

Table 7.7. Epiphytic lichen species recorded on *Pinus sylvestris* and *Betula pubescens* in the vicinity of the experimental site at Whim Moss, showing presence on trunks and twigs, foliose and crustose habits, acidophyte or nitrophyte indicators according to van Herk (2002).

Whim moss	Trunk	Twig	Mac/crust	N or A
Bryoria fuscescens		X	M	A
Cladonia spp	X		M	A
Fuscidea lightfootii	X		C	
Green crust		X	C	
Hypogymnia physodes	X	X	M	A
Hypogymnia tubulosa		X	M	A
Lecania cyrtella	X		C	
Lecanora chlarotera	X	X	C	
L. pulicaris	X		C	A
L. symmicta		X	C	
Lepraria spp.	X	X	C	A
Parmelia sulcata		X	M	
Platismatia glauca	X	X	M	A
Pseudevernia furfuracea		X	M	A
Scoliciosporum chlorococcum		X	C	
Usnea hirta		X	M	A

Nitrophyte and acidophyte indicators

Nitrophytes were absent from *Pinus* at both sites and present on twigs of *Betula* at low frequency in sampling site 1 (Tables 7.7 and 7.8). Acidophyte values were higher on pine trunks at sampling point 1, but higher on twigs of both *Pinus* and *Betula* at sampling point 2, about 100 m from the source, indicating loss of acidophytes in the vicinity of the ammonia source. Bark pH of twigs and trunks was lower at sampling site 2.

Table 7.8. Whim moss sampling sites S1 and S2 on *Pinus sylvestris* (Ps) and *Betula pubescens* (Bp), showing bark pH and acidophyte (AV) and nitrophyte (NV) values.

Whim	S1 Bp	S2 Bp	S1 Ps	S2 Ps
Bark pH			4	3.6
Twig pH	5.5	4	5	4.3
AV trunk			27	23
Diversity trunk			0.58	10
AV twig	0.25	4.5	1.3	2.5
NV twig	0.25	0	0	0
Diversity twig	2.5	2.7	1.4	2

Ellenberg values

Ellenberg values for N on *Pinus* trunks show a lower value in sampling site 2 (Average AV in site 2 = 9.3 StD 2.6, in site 1 11.2 StD 2.2), due to presence of *Lepraria* species on all trunks in sampling site 1 and its absence in S2.

7.3.4. Dunslair heights

The former CEH atmospheric monitoring site at Dunslair heights was visited as there were no trees present in the vicinity of the NH_3 recording station at Bowbeat Hill. At Dunslair heights there was a young *Larix* plantation where twigs were sampled from the summit at c. 607 m down a ride on a steep incline to the SW.

Results

Epiphytic lichen species found on *Larix* twigs at Dunslair heights are shown in Table 7.9.

Table 7.9. Epiphytic lichen species recorded on sampled *Larix europaea* plantation downhill from a former CEH ammonia recording station, showing presence on twigs, foliose and crustose habits, acidophyte or nitrophyte according to van Herk (2002).

Dunslair	Twig	Macro/crust	N or A
Bryoria fuscescens	X	M	A
Green crust	X	C	
Hypogymnia physodes	X	M	A
Hypogymnia tubulosa	X	M	A
Lecanora chlarotera	X	C	
L. symmicta	X	C	
Parmelia sulcata	X	M	
Platismatia glauca	X	M	A
Pseudevernia furfuracea	X	M	A
Scoliciosporum chlorococcum	X	C	
Usnea hirta	X	M	A

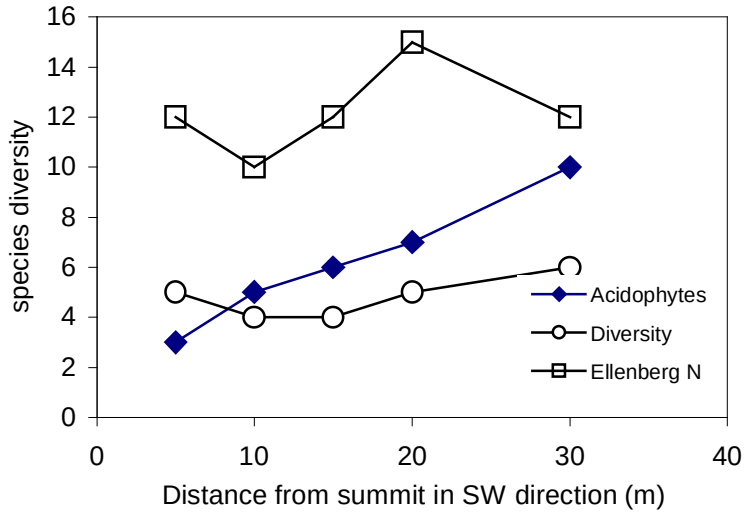

Figure 7.9. Lichen mean values at Dunslair showing an increase in acidophyte value and Ellenberg N on *Larix* twigs down slope from former monitoring station.

No nitrophytes were recorded at this site, but acidophytes were abundant and showed an increase in species diversity with increasing distance from the summit (Figure 7.9). In addition sensitive species *Bryoria fuscescens* was only found at sampling site 5.

The Ellenberg scale for N also showed a tendency to increase with distance from the summit. This may have been due to topography and the possibility of a more sheltered situation downwind of the summit and changes in available nutrients down slope. Bark pH was not done for this site.

7.4. Discussion

7.4.1. Site selection

Piddles Wood was the only site that fulfilled the criteria for epiphytic lichen sampling in that:
a) trees of same species were present throughout the transect

b) sufficient numbers of trees of the same species could be sampled for trunks and twigs at all stations except site 2 where only one oak tree was present among young regenerating *Fraxinus* trees.

The only difficulty with this site was the scale of the transect as within a 20m section it was difficult to find 5 appropriate trees.

At Happendon Wood tree species varied across the transect, with tree age and woodland structure varying from dense pine plantation to open wood pasture and from exposed well drained slope to wetland area with sphagnum bog. In this situation data on other environmental variables including humidity and slope is needed to determine contribution of factors other than measured N pollutants to species distribution. Only 2 sampling sites had oak trees and these were in very different habitat conditions. However the loss of acidophyte species on twigs showed that AV was negatively correlated with increasing N in all forms, but that in this environment nitrophytes were infrequent on acid-barked trees. The other 2 sites at Whim Moss and Dunslair heights were not suitable for transects but used to investigate lichen diversity in monitored atmospheric conditions. Both sites are naturally acidic sites with conifers that would only develop nitrophytes in extreme conditions. The survey showed that nitrophytes were absent on conifers in both sites. However the loss of acidophytes was observed in the vicinity of the experimental plot at Whim Moss, confirming the deleterious affect of NH_3 on natural acidophytes prior to the appearance of nitrophytes.

In comparing the methodology it is clear that the replacement of acidophyte communities by nitrophytic ones on naturally acid barked trees is associated with increasing NH_3 concentrations, often at low levels, and with increasing bark pH. The use of widespread indicator species to define these communities allows the building up of comparative data, and its testing against other environmental factors (van Herk, 1999, 2002). However it is also apparent that sensitivity to NH_3 may vary across a species range and that indicator species used to construct AV and NV values need to be evaluated in a UK context against atmospheric monitoring of NH_3, NO_x and total N deposition.

7.4.2. Use of acidophyte, nitrophyte and Ellenberg values for lichens

In the Netherlands van Herk (1999, 2002) used multivariate analysis to determine nitrophyte species that were positively correlated with atmospheric NH_3 and acidophyte species that were negatively correlated with atmospheric NH_3. Ellenberg scores for lichens were developed by Wirth (1992) for a range of conditions including nutrient levels, moisture and toxi-tolerance. The latter scores were devised to assess species sensitivity to atmospheric pollutants contributing to acidification such as SO_2 and NO_x. Ellenberg scores were based on expert opinion of species distribution in continental Europe and shown to correlate well with measured data (Ellenberg, 1992). However their validity in areas outside central Europe has not been tested for lichens (Wirth, 1992). Our results show that the nitrophyte scores and the Ellenberg N scores produce very similar results and that these are well correlated with atmospheric NH_3. Although further analysis of a UK wide data set may refine indicator species values indicator species in this category are widespread throughout Europe. As shown at Piddles Wood the Ellenberg toxi-tolerant scale is not appropriate for assessing loss of sensitive acidophyte communities. The use of van Herk acidophyte indicators is appropriate for use in areas where N deposition is threatening acidophyte communities but the components of the indicator list have been assessed for the Netherlands and not for oceanic conditions of western and northern Britain. In these areas our results are not well correlated with NH_3 concentration, although in all cases there has been a loss of acidophyte species. Factors influencing distribution of acidophyte species such as rainfall, occult precipitation and altitude, need to be taken into account. Multivariate analysis of species and environmental factors would allow us to assess an indicator list for Britain.

7.4.3. Lichens on twigs and trunks

The results of all surveys have shown that lichens on twigs are better correlated with atmospheric conditions than lichens on trunks. This is due to the use of substrates of the same age and usually at roughly the same habitat conditions, as only accessible twigs are normally used. Lichen communities on trunks of acid barked tree species have been used successfully in the Netherlands in areas that were formerly lichen deserts owing to SO_2 deposition. In this situation nitrophyte communities are able to colonise exposed bark. However, in areas that were not affected by high SO_2 deposition, nitrophytes are slow to invade established lichen communities that are characteristic of former conditions. Lichens are often long lived and nitrogen is rarely lethal so that communities on trunks may be relics and often contain species that are indicators of ecological continuity. This is important in sites of conservation importance but results show that lichens on twigs are able to provide an early warning system of changes that may take some time on older substrates.

7.4.4. Bark pH

Although bark pH can be usefully used along transects of the same species, it is not expected to be appropriate as an indicator of the status of the lichen community across the UK, particularly due to the huge variation between and within species across the climatic conditions of the UK. The issues related to the UK wide application of bark pH measurements are addressed in Section 11. Nevertheless, it is extremely useful to measure trunk and twig bark pH in parallel to assessing lichen biodiversity, as this helps to relate the observed changes to chemical conditions of the substrate. At Piddles Wood (as at the Scottish farm study site, Sutton *et al*. 2004a), twig pH increased with increasing NH_3 (i.e. closer to the farm), with twig pH values being consistently higher than trunk pH values. This is consistent with the preference of nitrophytes for high pH substrates, suggesting that the effect of NH_3 may be (at least in part) mediated through ammonia increasing the pH of bark. The exception to this effect was noted for the highest NH_3 concentration site at Piddles wood, where the trunk pH decreased. This effect was also seen adjacent to the farm at Earlston (Sutton *et al*. 2004a), and may be due to either nitrification of deposited NH_3 or deposited N rich particles on the bark of trunks closest to poultry farm buildings (i.e. those within 5-10 m from source).

At Happendon Wood, there was no clear relationship between bark pH and distance from the road sources or NO_2 and NH_3 concentrations. This may be due to the generally lower range of pollutant exposure, making it harder to detect a clear signal. By contrast, the values for trunk pH were in general lower than for twig pH, which is consistent with the result at Piddles Wood and Earlston. Also the pH values were in general lower than for Piddles Wood, reflecting the lower N air pollution levels at Happendon Wood. The clear differences seen at Happendon Wood between *Pinus sylvestris* and *Quercus* demonstrate the importance of using transects with a single tree species wherever possible.

7.5. Conclusions

- Although there is obvious variation in the results from different tree species and different locations in the UK, when all data is expressed as AV-NV values the relationship between loss of acidophyte values and increase in nitrophyte values operates at all sites. Further refinement of indicator species for use in the UK will also reduce variation between sites.

- Epiphytic lichen communities of twigs are strongly correlated with NH_3 concentrations whereas lichen communities of trunks may carry relics of previous conditions.

- Loss of acidophyte communities is occurring at lower levels of NH_3 concentrations than invasion of nitrophytes.

- Application of epiphytic lichens as indicators of NH_3 concentrations is appropriate where acid-barked tree species of the same species are present and where there is some habitat homogeneity.

- The negative correlation of AV of twigs with all forms of monitored N deposition suggests a combined effect of N pollutants.

- Further testing of acidophyte indicators across the UK in a range of climatic and topographical conditions is necessary. Bark pH can be used on the same tree species in local situations as an indicator of increasing NH_3 concentrations.

8. Synthesis of the intensive sites study

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8.1. Introduction

The biomonitoring methods tested in parallel at the intensive sites are directly compared in this section. The objectives are to a) compare the robustness and practicality of the individual methods and b) using the two sites with gradient measurements, determine if there is a threshold N deposition value which can be assigned from the individual biomonitoring methods, to indicate that N is having a significant effect on integrity of the ecosystem under investigation.

8.2. Comparison of robustness and practical application of the biomonitoring methods

8.2.1. Piddles Wood (agricultural point source)

The summary Table 8.1 demonstrated that all the biomonitoring methods applied at Piddles Wood (the agricultural point source site with an N deposition range 1000-25 kg N ha^{-1} y^{-1}), showed that current N deposition is impacting across the site. All the applied methods were considered robust and could therefore be applied at other conservation sites with an identified strong point source attribution.

8.2.2. Happendon Wood (M74 motorway site)

The Ellenberg Index method (and modified acidophyte/nitrophyte index) was considered suitable for gradient studies at a motorway site (Happendon Wood) with an N deposition range of 12-23 kg N ha^{-1} y^{-1}. This is an important result because it shows the method is applicable and relatively robust despite a complex mosaic of habitats and species along the gradient away from the M74 motorway. Although, the pleurocarpous mosses did not show a strong relationship with tissue N content and N deposition, the relationship with N deposition and soluble NH_4-N concentration was stronger (R^2=0.51). This suggests that there is a trend of increasing foliar N concentration with increasing N deposition and indicates that the use of soluble NH_4-N concentration method was considered suitable even at this N deposition range. It would be interesting to apply this method to motorway sites with a greater NH_3 and N deposition range. In contrast, there was a strong relationship with soluble NH_4-N concentration and N deposition along the gradient away from the M74. Therefore, the soluble NH_4-N method using pleurocarpous mosses is considered robust.

The standardised grass biomonitoring method showed that there was a good relationship with biomass accumulation and distance from the M74 in relation to total N deposition. However, measurements of foliar N concentration (both tissue N and soluble NH_4-N) measurement or total foliar N inventory did not detect such relationships with N deposition. Overall, this method is considered suitable under specific conditions, as it was suspected that the exposure period of 12 weeks was insufficient to detect N impacts particularly through the observed exposure to NH_3, NO_2 and total N deposition rates at this site, but the method could be applicable at sites with higher N or exposed for a longer period.

8.2.3. Auchencorth Moss and Bowbeat Hill

All the applied methods showed that at these sites dominated by diffuse wet N deposition at N inputs of 12-25 kg N ha^{-1} y^{-1}, it is difficult to measure N impacts both over the short-term using standardised grass biomonitors or the longer-term using Ellenberg or tissue N content and soluble NH_4-N concentration methods. The site with larger N deposition (Bowbeat Hill) actually had smaller total foliar N than the site with lower N deposition (Auchencorth Moss). By contrast, the soluble foliar NH_4-N measurement gave a larger value at the high wet deposition site, but this was not statistically significant, indicating the need for more replication for such comparisons with only a modest difference in N inputs. The development of Acidophyte/nitrophyte index for higher plants may have some scope in detecting potential changes in species composition and therefore may be applicable to such monitoring methods, particularly with the conservation agencies, site condition monitoring system.

Long-term N impacts therefore, may be able to be detected at conservation sites with a potentially higher diffuse wet N deposition, than that experienced in the sites under this project. This is illustrated by the results from Whim Moss, where higher rates of wet N deposition were compared.

8.2.4. Whim Moss

The application of the biomonitoring methods at CEH's N manipulation site allowed the methods to be applied under field conditions at a range of NH_3 concentrations and wet N deposition. The methods employed demonstrated detectable N impacts on pleurocarpous moss (*H. jutlandicum*) exposed along a NH_3 gradient. Within the wet treatments, 32 and 64 kg N ha^{-1} y^{-1} (applied as either NH_4Cl or $NaNO_3^-$) N impacts were detectable in the moss species (*H. jutlandicum*) analysed, with increases in both tissue N content and soluble NH_4-N concentration after 2 years exposure. However, no such effects were found in the lower wet N deposition treatments (16 kg N ha^{-1} y^{-1}) of either N form. This confirms that the foliar N biomonitoring methods are robust and applicable at sites with diffuse N inputs but are deposition dependent i.e. requiring N deposition, which are possibly > 25 kg N ha^{-1} y^{-1}. As the standardised grass biomonitoring (*D. flexuosa*) plants did not show any N impacts either associated with tissue N content or soluble NH_4-N concentrations after 12 weeks of exposure, this method was therefore classified as being not suitable. It is suspected that the total N deposition of ~1.4, 5 and 10 kg N ha^{-1} y^{-1} respectively for the 3 treatments (8, 32 and 64 kg N ha^{-1} y^{-1}) over the exposure period were insufficient for detection of N impacts by the grass biomonitors. Therefore, applications of standardised grass biomonitors methods are possible at diffuse wet N sites, but it would require a longer exposure period of 6-12 months.

Table 8.1. Robustness of the N biomonitoring methods tested in parallel at the four intensive sites.

Source attribution	Agricultural point source	Motorway	Lowland vs. Upland moor	Manipulation study	Manipulation Study
N source	NH_3	NO_2 & NH_3	NH_4^+, NO_3^-	NH_3	NH_4^+, NO_3^-
Wet or dry dominated	Dry	Dry/wet	Wet	Dry	Dry/wet
Ellenberg Index	R	S	NS	Not tested	Not tested
Pleurocarpous Moss % N	R	SC	NS	R*	R*
Pleurocarpous Moss NH_4-N	R	R	NS#	R*	R*
Stand. plants *D. flexuosa* % N	R	SC	NS	S	S
Stand. plants *D. flexuosa* NH_4-N	R	SC	NS	S	S
Stand. plants *D. flexuosa* Biomass	R	R	NS	NS	SC*
Lichen indicators: Trunk	R	SC	SC	SC	SC
Lichen indicators: twig	R	SC	SC	SC	SC
Lichen bark pH	R	SC	SC	SC	SC
Lichen twig pH	R	SC	SC	SC	SC

R=robust, good strong method

S=suitable:

SC= suitable under specified conditions:

NS=not suitable

R* robust with ambient NH_3 concentrations (>5 μg m^{-3}) and N deposition (16-32 kg N ha^{-1} y^{-1}). The NH_3 concentrations and N deposition threshold values are based on the data from the 3 month study. These could vary in a study with a longer exposure period.

This comparison did give larger foliar NH_4-N at Bowbeat, but it was just not significant with the data collected. It is suspected that a larger sample size would give a better indication of the relationship between soluble NH_4-N and wet N deposition.

8.3. Comparison of the practical application of the biomonitoring methods

Table 8.2 summaries the practicality of the biomonitoring methods tested in this study for application by the conservation agencies as additional tools for assessing N impacts on the condition and integrity of designated sites. The **Ellenberg Index** could be used as both an indicator of N impact and also as a useful description of the current species composition of the site. This method however requires botanical expertise and may not always be applicable for Condition Standards Monitoring (CSM) depending on the level of expertise of the officer conducting the CSM assessment. The application of the simplified Ellenberg Index using the acidophyte/nitrophytes may be applicable at specific sites. If a site was considered to be impacted by N, then a specialist contractor could be used to carry-out an additional survey. **The total N content and the soluble NH_4-N methods** both require some bryophyte identification skills and the expenditure on chemical analysis. The current cost per sample for per analysis is £10-£15 excluding processing the sample (cleaning, preparation) at £20 per sample. **The standardised grass transplants method** requires some botanical expertise but is relatively cheap to set-up. The cost per six-plant tray would be approximately £30 including

materials and grass biomonitor propagation. There would be the additional chemical analysis costs if foliar N concentrations were required (see above).

Table 8.2. The practicality of the N biomonitoring methods tested in parallel at the four intensive sites

Source attribution	**Agricultural point source**	**Motorway**	**Lowland moor**	**Upland moor**	**Manipulation Study**
N source	**NH_3**	**NO_2 & NH_3**	**NH_4^+, NO_3^-**	**NH_4^+, NO_3^-**	**NH_3, NH_4^+, NO_3**
Wet or dry dominated	**Dry**	**Dry/wet**	**Wet**	**Wet**	**Dry/wet**
Ellenberg Index	BE	BE	BE	BE	BE
Pleurocarpous Moss % N	S*	S*	S*	S*	S*
Pleurocarpous Moss NH_4-N	S*	S*	S*	S*	S*
Stand. plants *D. flexuosa* % N	EP	EP	EP	EP	EP
Stand. plants *D. flexuosa* NH_4-N	EP	EP	EP	EP	EP
Stand. plants *D. flexuosa* Biomass	EP	EP	EP	EP	EP
Lichen indicators: trunk	BE	BE	BE	NA	BE
Lichen indicators: twig	BE	BE	BE	NA	BE
Lichen bark pH	S*	S*	S*	NA	S*
Lichen twig pH	S*	S*	S*	NA	S*

S* simple, but requires chemical analysis of samples.
BE: requires significant botanical expertise:
EP: requires some equipment/standardised plants
NA: not applicable

If only using a limited number of macro-lichens the level of expertise required would be significantly reduced.

8.4. Comparison of biomonitoring methods use in the assessment of N impacts along the NH_3 gradient at Piddles Wood, Dorset

The biomonitoring methods tested in parallel along a NH_3 gradient from an agricultural point source are compared in Table 8.3. Assessments of N impacts are made for each method at the different distances along the transect to directly compare the responses of the individual methods.

The results show that the Ellenberg Index, foliar N concentration methods and the standardised grass bioindicators all strongly indicate N impacts within 5-50 m from the point source/ 101-17 μg NH_3 m^{-3}/ 1061-188 kg N ha^{-1} y^{-1}. Flora changes were detected between 50 and 100 m from the poultry unit changing from nitrophytic dominated species to acidophytic species the further away from the source. Whereas, the foliar N concentration measurements for pleurocarpous mosses indicate that N is impacting at 250 m from the poultry unit. This would suggest that the foliar N content and soluble NH_4-N concentrations are more sensitive measures of N impacts, the response time could be quicker, or that the critical limit has been set to a more sensitive value. While Ellenberg Index gives a measure of current species composition and status of the site within the NVC classification, the foliar N measurements show the potential for change and damage to the 'health' of the habitat before such shifts in

species composition are detected. The standardised grass biomonitors demonstrated shifts in tissue N content and biomass accumulation in direct response to N exposure along the length of the transect, thus indicating direct N effects. However, the soluble NH_4-N concentrations for the grass biomonitors indicate that this particular method could detect small changes in N impacts, thus indicating a more sensitive method for such sites.

There is substantial uncertainty at this present time as to the setting of critical values for acidophyte and nitrophyte lichens and therefore further assessment is required for this potential method. However, an initial value threshold has been set for where acidophytes equal the nitrophytes, then where AV>NV then the lichen flora is acidophyte dominated and where NV>AV then the flora is nitrophyte dominated. With these values there is an inconsistency between the results for twig lichens vs. trunk lichens. Twig lichens suggest that the site is impacted by nitrogen at all positions in the transect (nitrophyte dominated); while the trunk lichens suggest that the site is only impacts at the site closest to the farm. This indicates a need to refine the critical values for lichens. In particular, the value to be set may depend on the "target condition" for a particular habitat. Comparison with the UK scale lichen assessment, suggests that the critical value for twigs of AV-NV<0, equates to a critical value for trunks of AV-NV<+10. Using this critical value for trunks would suggest that all sites on the Piddles Wood transect are exceeded for nitrogen, consistent with the twig lichen data.

Table 8.3. Comparison of the biomonitoring method used at Piddles Wood adjacent to a point NH_3 source.

N deposition (kg N $ha^{-1}y^{-1}$)	**1061**	**337**	**188**	**31**	**26**
Distance from $_{NH3}$ point source (m)	**5**	**20**	**48**	**100**	**250**
NH_3 concentration (μg m^{-3})	**101**	**31**	**17**	**2**	**1.5**
Ellenberg Index (presence) (Mean N index >5)	Yes	Yes	Yes	Yes	No
Higher plants (presence) modified for (NV> AV)	Yes	Yes	Yes	No	No
Higher plants (cover) modified for (NV>AV)	Yes	Yes	Yes	Yes	Yes
Mosses % N (> 1.3% N)$^{+}$	Yes	Yes	Yes	Yes	Yes
Mosses NH_4-N (> 6 μg g^{-1} FW)$^{++}$	Yes	Yes	Yes	Yes	Yes
S.G.Bioindicators % N (>1.0% N)^{0}	Yes	Yes	Yes	Yes	N/A
S.G.Bioindicators NH_4-N (> 6 μg g^{-1} FW)^{00}	Yes	Yes	Yes	No	N/A
S.G.Bioindicators Biomass (N impact)	Yes	Yes	Yes	Yes	N/A
Lichen indicator values – trunks *Quercus* (NV>AV)*	Yes	No	No	No	No
Lichen indicator values – twigs *Quercus* (NV>AV)*	Yes	N/A	Yes	Yes	Yes

* If Acidophyte Value (AV) is less than Nitrophyte Value (NV) then nitrophytes set as dominating. There is uncertainty in setting this critical value, and a positive value of AV-NV may be more appropriate.

+The concentration of > 1.3% N was the mean total N content using the UK extensive sites pleurocarpous moss data. It was also the value set to detect N impacts in Section 10 for the determination of mean habitat total N content, which was derived by expert judgement.

++The value of 6 μg g^{-1} FW was the mean NH_4-N concentration using the UK Extensive sites pleurocarpous moss data.

The concentration of > 1.0% N was the mean total N content for the *D. flexuosa* grass plants at Whim Moss. This concentration was taken as a threshold value and above this threshold value it was estimated that N was impacting on the site.

**The value of 6 μg g^{-1} FW was the mean concentration measured in the control treatment *D. flexuosa* plants at Whim Moss. This concentration was taken as a threshold value and above this threshold value it was estimated that N was impacting on the site.

Standardised grass bioindicators (S.G.Bioindicators).

8.5. Comparison of biomonitoring methods for assessment of N impacts along a transect from the M74 motorway (Happendon Wood, south Lanarkshire)

Table 8.4. Comparison of the biomonitoring method used at Happendon Wood.

N deposition (kg N $ha^{-1}y^{-1}$)	**70.7**	**55.3**	**69**	**35**	**29**	**31**
Distance from NH_3 point source (m)	**10**	**20**	**38**	**150**	**200**	**250**
NH_3 concentration (μg m^{-3})	**1.3**	**0.5**	**0.9**	**0.5**	**0.4**	**0.5**
NO_2 concentration (μg m^{-3})	**13.4**	**11.5**	**14.0**	**6.3**	**5.6**	**4.8**
Ellenberg N index (NI>5)	Yes	Yes	No	No	No	No
Higher plant (NV>AV)	Yes	Yes	Yes	No	No	No
Mosses % N (> 1.3% N)$^{+}$	Yes	Yes	No	Yes	No	No
Mosses NH_4-N (> 6 μg g^{-1} FW)$^{++}$	Yes	Yes	Yes	Yes	Yes	Yes
S.G.Bioindicator % N (>1.0% N)^{0}	Yes	Yes	No	N/A	No	Yes
S.G.Bioindicator NH_4-N (>6 μg g^{-1} FW)**	No	No	No	N/A	No	No
S.G.Bioindicator Biomass (N impacting)	Yes	Yes	Yes	N/A	No	No
Lichen indicators values – trunks *Pinus*	Yes	Yes	Yes	Yes	Yes	Yes*
Lichen indicator values – twigs *Pinus*	Yes	Yes	Yes	Yes	Yes	Yes
Quercus trunks and twigs					N	N

N/A not applicable: standardised grass biomonitors were not exposed at this location.

Standardised grass (S.G.)

* The 150 m point was an additional point added after 6 weeks into the study (Section 3).

The concentration of > 1.0% N was the mean total N content for the *D .flexuosa* grass plants at Whim Moss. This concentration was taken as a threshold value and above this threshold value it was estimated that N was impacting on the site.

**The value of 6 μg g^{-1} FW was the mean concentration measured in the control treatment *D. flexuosa* plants at Whim Moss. This concentration was taken as a threshold value and above this threshold value it was estimated that N was impacting on the site.

+ The concentration of > 1.3% N was the mean total N content using the UK extensive sites pleurocarpous moss data. It was also the value set to detect N impacts in Section 10 for the determination of mean habitat total N content, which was derived by expert judgement.

++ The value of 6 μg g^{-1} FW was the mean NH_4-N concentration using the UK Extensive sites pleurocarpous moss data.

Table 8.4 shows that soluble NH_4-N concentrations in the pleurocarpous mosses were above the threshold value at all positions along the 250 m transect. This would suggest that the mosses were responding predominantly to the wet N inputs rather than the NH_3 and NO_2 concentrations, which were relatively low throughout the transect. The total tissue N content

in the mosses reduced with decreased N deposition associated with distance from the M74 motorway, with N content below the threshold value at 200 and 250 m. However, the soluble NH_4-N method is sensitive enough to detect N input changes in the range 12- 23 kg N ha^{-1} y^{-1} in pleurocarpous moss tissue.

The tissue N content in the standardised grasses provided similar results to those for the moss, with reductions in N the further away from the motorway. Again the grass bioindicators exhibited no impact of N at 150 and 200 m from the motorway. A similar result was also demonstrated with the use of the modified Ellenberg acidophyte/nitrophyte Index, which showed that N deposition was not affecting the site at 200 and 250 m from the motorway. Soluble NH_4-N concentrations in the standardised grasses were not affected by the pollution gradient.

Using the modified Ellenberg acidophyte/nitrophyte Index shows that N deposition is not affecting the site at 200 and 250 m from the M74. The total N content in the mosses reduced with distance/ N deposition along the transect. The low N contents at 38 m are not fully understood but could be due to habitat shading at this position. The N contents in the pleurocarpous mosses were below the threshold value at 200 and 250 m from the M74. Using the N contents as bioindicators this result would again indicate that the M74 emissions are not impacting on the site at a distance of 200 m and 250 m from the motorway.

The N content for pleurocarpous mosses gave a similar indication of impact as did the N contents of the standardised grasses, whereas, the soluble NH_4-N concentrations in the standardised grasses were not affected by the pollution gradient. These results suggest that mosses are a better species for use with the soluble NH_4-N concentration method than *D. flexuosa* as they appear to be more sensitive to increases in N than the grass.

8.6. Tissue N content and soluble NH_4-N concentration along a gradient of pollutant from a point source

The use of gradient studies away from a known N source (Pitcairn et al. 2003, Sutton *et al*. 2004a) have shown clear responses in tissue N content, soluble NH_4-N concentration and lichen diversity with NH_3 concentration and N deposition. However, it is important in studies of this type to have as uniform a vegetation type as possible along the N gradient. The presence of the same target individual species at each of the distances along the transect is advantageous to the interpretation of the data and determining threshold distances from the source. The distance (m) of any potential gradient from the source is also important. As it is estimated that for mosses the optimum distance from source is up to 250 m away from the N point source and greater than 300 m for lichens with the current study indicating a signal in the lichen diversity of twigs at 250 m from the poultry unit at Piddles Wood.

Additional measurements NH_3 measurements (08/06/04-21/07/04) showed that the concentration had decreased from 1.96 μg m^{-3} to 1.46 μg m^{-3} between 100 m and 250 m from the point source.

8.7. Conclusions

- A comparison of the biomonitoring methods at Piddles Wood (agricultural NH_3 point source) demonstrated that they were all robust enough for use by the conservation agencies and SEPA at sites with a defined point source.

- The results form the other intensive site with a defined source, the M74 motorway at Happendon Wood, showed that Ellenberg was suitable, and that the soluble NH_4-N

method was robust, whilst the other methods were considered to be only suitable under specific conditions (i.e. at sites exposed to larger ambient N concentrations).

- None of the biomonitoring methods tested at the two sites dominated by diffuse N deposition (Auchencorth Moss or Bowbeat Hill) were considered robust enough to detect changes in N under a relatively small difference in deposition (10 kg N ha^{-1} y^{-1}). Therefore in order to detect a signal dominated by wet deposition using biomonitoring would require greater replication and/or a greater difference in N deposition.

- Ellenberg N Index and modified Acidophyte/nitrophyte Index for higher plants give a measure of current species composition and the extent to which nitrophyte species dominate a site. They confirm the current status of a site within the NVC classification and can show areas where change has already occurred. With botanical knowledge, the Ellenberg index is straightforward to apply and has the added benefit of providing a species list for the site. The Acidophyte/nitrophyte Index could prove a simpler and more robust method of determining site condition in direct relation to N impacts. This method could be further developed to use key species thus making it simpler to apply with limited botanical training.

- Foliar tissue N measurements are a sensitive indication of N impacts and show the potential for change and damage to the 'health' of the habitat. The results indicate the need to use the data from several indicator methods when making site assessments. In particular it is recommended to combine different method types, e.g.
    - A method such as Ellenberg for lichen twig test could be applied to give the current effect of N deposition at a site in terms of visible changes (loss or reduction in desirable species, gain or increase in undesirable species).
    - A second method such as foliar N or standardised grass plants could be use to give an indication of the load of N on the ecosystem and the potential for translation into changes in species health and diversity.

- Additional methods may be applied, appropriate to the context of the assessment (e.g. screening or full assessment, and approaches to combining nitrogen indicator methods are further described in Sections 14-16.

9. Extensive UK scale study: biomonitoring methods

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9.1. Introduction

Having tested the simplified biomonitoring methods in parallel at the intensive sites, the second phase of the project was to apply selected methods across the whole of the UK. The aim was to assess the extent of relationships and to determine the robustness and consistency of the methods in relation to assessing the N impacts at statutory nature conservation sites. In order to carry out this project at the UK scale and at a reasonable cost it required a) the involvement of the staff from both the conservation and environment agencies and b) the methods to be applied by trained but non-specialist personnel from the agencies. It was hoped that the involvement of the staff from the agencies would raise awareness of the problems associated with N based pollutants and provide appropriate training, which could be beneficial to both staff and the agencies at a local scale. The provision of basic training in the identification of lichen and moss along with the implementation of the lichen diversity assessment and moss sampling protocols was an integral part of study. Thirty-two sites distributed throughout the UK were selected for testing of the simplified methods, identified in Sections 6 and 7. The site selection criteria are fully discussed in Section 9.2.

The tissue N content (% dry weight) method using mosses has been extensively used at localised agricultural-based N point sources (Pitcairn *et al*. 1998, 2003) and to a limited extent in studies looking at more local, regional and national level associated diffuse N sources, in particular as wet N deposition (Pitcairn *et al*. 1995).

Foliar N content has been studied in parallel with certain other bioindicator methods (including Ellenberg Index and amino acids) at two localised agricultural sources (Pitcairn *et al*. 2002, 2003). However, the total foliar N content method has not been tested on a UK scale in parallel with other bioindicator methods.

Soluble foliar ammonium concentration is a recently developed biomonitoring method, which has only been tested at the local field scale along a gradient from an agricultural point source (Sutton *et al*. 2004a), as well as testing the foliar content of agricultural and woodland plant species (Hill *et al*. 1999, Loubet *et al*. 2002).

The lichen diversity method has until now been applied at the local scale in the UK by expert lichenologists (Wolseley and James 2002a; Sutton *et al*. 2004b). The method of distinguishing nitrophyte and acidophyte lichens was developed originally for the Netherlands by sampling on tree trunks (van Herk 1999) and has since been extended by Wolseley and Pryor (1999) for sampling lichens on twigs. In the study downwind of a farm in Scotland, Sutton *et al*. (2004a) showed that it was not necessary for an assessment to identify all lichen species in order to conduct an assessment, but that a basic screening could be conducted by considering only the major, easy to identify macrophytic lichens. The present study assesses the potential application of such a simplified method using non-expert lichenologists to conduct the field recording.

The objectives of the Extensive UK study was to test in parallel, the simplified biomonitoring methods and assess their robustness, reproducibility and applicability as an additional tool for the conservation agencies to use in their condition monitoring assessment of statutory sites

and to assess potential N impacts on the long- term integrity. Such methods could also be employed by the environment agencies in order for them to conduct environmental impact assessments associated with point-source polluters under regulatory control. These N biomonitoring methods could be applied when N-based pollutants have been identified as potentially impacting on the designated site or could affect the long-term integrity of the site.

9.2. Methods

9.2.1. Site selection of UK sites

The selection of UK sites was critical to the success of the study. The key requirement was that robust estimates of air pollutant exposure were readily available, as additional measurements were beyond the scope of this study. Wet deposition estimates could be estimated with reasonable certainty (apart from in complex hill terrain), but it is important to have measurements of NH_3 concentrations. At the same time, given that SO_2 has in the past been a key determinant of lichen biodiversity, in order to distinguish NH_3 and SO_2 effects, it was highly desirable to use sites where SO_2 concentrations are known.

Although it was not essential that the biomonitoring measurements were conducted on statutory nature conservation sites, it was desirable that at least a fraction of the sites were designated in order to make the link to possible impacts associated with the integrity of these key sites. A large number of NH_3 monitoring sites in the UK were used in this study, but not all of these had SO_2 estimates. As one of the simplified methods to be tested was epiphytic lichen diversity, it was important that a large number of the sites had appropriate tree species present. However, it was recognised that not all the biomonitoring methods could be tested at all sites, therefore appropriate method selection had to be conducted for each site.

The site selection criteria included:

1. Availability of complete air monitoring of N and S in air and precipitation.
2. Appropriate vegetation nearby with trees and bryophytes.
3. Covers a wide range of N concentrations and deposition including clean sites and sites with high wet deposition and high dry N deposition.
4. Relevant nature conservation interest at the site.
5. Availability of a local conservation agency and/or environment agency officer willing to participate in the study and conduct the field sampling.
6. Representative coverage in different UK devolved regions, particularly Scotland and Northern Ireland (due to reasons of project co-funding).

If there was not a full range of air monitoring available (1) then the next priority requirements were:

7. NH_x monitoring available and precipitation chemistry (not but SO_2) plus criteria 2-5

Or:

8. Full air chemistry, plus 2, 3 & 5 but not a nature conservation interest

Or

9. NH_X monitoring available, but not SO_2 or precipitation chemistry and site in a lowland area# plus criteria 2-5.

Or:

10. Precipitation chemistry available and some NH_3 data available and sites in wet areas plus criteria 2-5

Or

11. NH_3 monitoring available in lowland area but not SO_2 and not wet deposition with wet deposition taken from UK precipitation maps.

Inevitably there was substantial balancing between the priorities, and in particular a number of sites were identified due to the availability of air monitoring data and their contribution to ranges of N deposition, even if the site was not of particular nature conservation interest.

Local officers were contacted either by the Steering group members from the appropriate sponsoring agencies or by CEH staff. The final selection of 32 sites for the testing of the simplified biomonitoring methods was done in consultation with the Project Steering Group. The sites, location and site officer are listed in Table 9.2. Figure 9.1 shows the distribution of sites throughout the UK.

Figure 9.1. Map of the UK showing the locations of the 32 extensive sites.

9.3. Training Course

A one-day training course was organised for those site operators who required basic instruction in lichen and moss species identification. The Natural History Museum (NHM) and CEH Edinburgh staff members ran the course on 25 May 2004 at CEH Edinburgh. The format of the course was a combination of Powerpoint presentations on lichen and moss sampling protocols and identification procedures followed by two lichen field identification sessions run by NHM. The aims of the course were to a) provide the site operators with the basic lichen and moss field identification skills required to carry-out the sampling procedures, b) to explain the protocols and c) distribute the sampling packs. Seventeen individuals representing fifteen of the thirty-two sites, who had a range of field identification experience, attended the course. Feedback from individuals on the course indicated that the course had been successful with the level and format having being pitched correctly.

In general, individuals were concerned about field identification of the epiphytic lichens but had a working knowledge or were more comfortable with the identification of the pleurocarpous moss species. The course allowed CEH and NHM staff to meet a number of the site operators, discuss informally their specific sites and sampling questions. Having had personal contact with the site operators also helped considerably in subsequent communications about a range of issues. Those individuals who did not require training were sent comprehensive instructions on the protocols with their sampling packs.

9.4. Field Officer sampling packs

SAMPLING PACK

The sampling pack was sent out to all those site operators, who had indicated that they did not require training, on 19 May 2004. The pack contents were contained in a cardboard postal box and included a letter giving background information on the project, sampling times and a list of contact names and addresses.

The contents of the pack were:

- Lichen sampling protocol (white sheets): see section
- Lichen results sheet (white sheet)
- Lichens on trunks and twigs site information recording form (light blue sheet)
- Lichens on trunks recording form (light green sheet)
- Lichens on twigs recording form (yellow sheet)
- Lichen identification key + A5 laminated sheet with 4 extra species (please keep both for your own use)
- Plastic quadrat ladder for lichen diversity
- Map pins for attaching ladder to tree trunk
- White paper bags for twig and bark samples and any lichen samples that require identification. Please label all packets with site, sample and date.
- Moss sampling protocol (white sheets)
- Moss sampling data sheet (peach sheet)
- Plastic bags for moss samples and any moss samples that require further identificatio Please label all packets with site, sample and date.
- Marker pen and Pencil
- Disposable gloves to be worn for collection of moss samples
- Self adhesive return address label

You will also need a hand lens, knife, compass and a measuring tape but we have not included these in the pack on the assumption that local agency offices would hold these.

The authors are grateful to the site officers for their efforts on our behalf and thank them for the general high standard of the data collection and sampling provided.

The individual site operators were requested to complete the lichen diversity survey and collect the lichen, bark and moss samples within a 45-day window (25 May-9 July 2004). Only two of the sites returned the completed data sheets and samples by the deadline date. This was due to other commitments on time and not a lack of enthusiasm for the project. In addition, data and samples from an additional site in Northern Ireland were also received. All 32 sites returned the sampling packs but unfortunately not all samples/forms were usable.

Table 9.1. Indication of the quality of data and samples from the 32 UK extensive study sites.

Tasks	Number of sites
Number of sites returning completed sampling packs by 9 July 2004	2
Number of sites returning completed sampling packs *	33
Number of sites returning completed lichen diversity data sheet	31
Number of sites returning maps /photographs of their sites	23
Number of sites returning completed twig lichen diversity data sheet	31
Number of sites returning a set of twig lichen measurements	29
Number of sites returning samples for bark pH measurements	29
Number of sites phoning CEH with questions/sampling problems	6
Number of sites returning completed moss data sheets	31
Number of sites returning the correct number of moss samples **	9
Number of moss sample bags with species incorrectly labelled	~ 15
Number of sites returning unusable moss samples	2
Number of moss sample bags containing a mixture of species	~ 50
Number of sites returning unusable lichen samples (twigs)	0
Number of sites returning unusable lichen samples (bark)	2

The original protocol stated that two different moss species should be collected from three areas at each of the sites. This was not always possible due to lack of availability of the two mosses at each of the three collecting areas. Fourteen of the sites sent back two or more different moss species, while the majority of the rest sent back only one species from the three specified areas within their site. The protocol provided clear instructions on the priority moss species for collection, in order to reduce the number of species used in the analysis. However, many different moss species were returned, which was principally due to difficulties of obtaining the correct number of samples/species due to within site variability in moss cover.

These findings indicate that it is possible to set sampling protocols, however, the application in the field is not always as straightforward.

Lichen samples were collected in labelled paper bags and dried, thus allowing lichen species identifications recorded by the surveyors to be checked at a later date by the Natural History Museum. Samples from twigs sometimes contained more species than were recorded on the diversity sheet due to small macro lichens being present. This could only be checked if samples were sent in.

Specimens collected for bark pH were also collected in labelled paper bags and dried. Lichen pH was measured at the NHM in November, 2004. Specimens from twigs were occasionally larger than the specified diameter and some were split when not collected with secateurs. These samples were discarded. However, most collectors sent plenty of twig samples to choose from.

9.5. UK Sites

Thirty-two sites were selected to test in parallel the simplified biomonitoring methods. The sites were distributed throughout the UK and comprised of four Welsh, nine Scottish, thirteen English and five Northern Ireland sites (See Figure 9.1 for site distribution and identification).

Table 9.2. List of UK sites, grid references and site officer responsible for survey/sampling.

Site no.	Site Name	Nat Grid Reference	Site officer
1	Cwmystwyth	SN772743	Ray Woods: CCW
2	Plynlimon	SN822841	Ray Woods:CCW
3	Dyffrn Mymbyr	SH695572	Alex Turner:CCW
4	Stackpole	SR983950	Bob Haycock : CCW
5	Eskdalemuir	NT235032	Chris Miles: SNH
6	Halladale	NC902488	Sandy Payne: SNH
7	Strathvaich Dam	NH347750	John Clayton, Ed. Turner: SEPA
8	Glensaugh	NO664799	John. Clayton, Ed. Turner: SEPA
9	Inverpolly/Knockan	NC187088	Jan Breckenridge:SNH
10	Edinburgh Centre	NT254738	Ian Leith, Netty van Dijk: CEH
11	Ariundle	NM835642	Brian Eardley: SNH
12	Glen Nant	NN014278	Janie Steele: FC
13	Wood of Cree	NX385715	Tommy Docherty: SNH
14	Bush	NT245635	Ian Leith, Netty van Dijk: CEH
15	Sherwood (Ladybower)	SK163905	Richard Pollitt: EN
16	Moorhouse	NY751334	Bart Donato: EN
17	Fenns' Moss B	SJ478368	Joan Daniels:EN
18	Stanford	TL858948	Peter Lambley:EN
19	Fressingfield	TM261759	Allison Collins:EN
20	Bedlington/Bedingfield	TM173684	Allison Collins:EN
21	Borrowdale	NY240135	Bart Donato: EN
22	Brown Moss	SJ563390	Chris Hogarth :EN
23	Llnclys Common	SJ273237	Chris Hogarth : EN
24	Wytham Wood	SP452083	Ron Porley: EN
25	Lullington Heath	TQ538016	Malcolm Emery: EN
26	Lough Navar	H074545	Melina McMullan: EHS
27	Glenmore Wood	H654608	Melina McMullan: EHS
28	Caldanagh Bog	D022205	Melina McMullan: EHS
29	Castle Enigan	J121322	Melina McMullan: EHS
30	Orritor	H768782	Melina McMullan: EHS
31	Redgrave and Lopham	TM050797	Andrew Excell: SWT
32	Yarner Wood	SX786789	Pat Wolseley: NHM

All NH_3 data was obtained from either current CEH monitoring as part of the National Ammonia Monitoring Network (NAMN) or from recent monitoring conducted at the individual sites as part of other CEH projects. The SO_2 data (annual mean concentrations) is from the national rural SO_2 and HNO_3 monitoring networks. Values from the mapped SO_2 concentration field (NEGTAP 2001) are also shown for comparison. The ammonia data highlighted the fact that the sites selected were representative of a wide range of UK NH_3 concentrations. The concentrations ranged from the lowest concentration at Ariundle, a wet N deposition dominated, Atlantic oak woodland in the western Highlands (0.04 $\mu g\ m^{-3}$) to the highest at Bedlington, in the dry N deposition dominated East Anglia (7.4 $\mu g\ m^{-3}$). This covers the full range of NH_3 concentrations as recorded in the NAMN.

Table 9.3. List of UK sites indicating habitat types and NH_3 data, mapped SO_2 concentrations and where available the SO_2 annual mean concentrations.

Site no.	Site Name	Habitat type	NH_3 concentration $\mu g\ m^{-3}$	Measured SO_2 concentration $\mu g\ m^{-3}$	Mapped SO_2 concentration $\mu g\ m^{-3}$
1	Cwmystwyth	Acid grassland	2.96	1.11	1.5
2	Plynlimon	Upland heath	0.60		1.5
3	Dyffrn Mymbyr	Wet acid heath	1.18		2.6
4	Stackpole	Lowland woodland	1.81		2
5	Eskdalemuir	Upland moorland	0.38	0.82	1.1
6	Halladale	Improved grassland	0.83		1.2
7	Strathvaich Dam	Upland moorland	0.18	0.23	0.6
8	Glensaugh	Upland moorland	0.35	0.79	1.1
9	Inverpolly/Knockan	Upland moorland	0.12		0.8
10	Edinburgh Centre	Parkland	2.5		16.6
11	Ariundle	Atlantic oak woodland	0.04		1.4
12	Glen Nant	Atlantic oak woodland	0.06		1.6
13	Wood of Cree	Atlantic oak woodland	0.12		1.5
14	Bush	Parkland	0.90	1.81	3.7
15	Sherwood (Ladybower)	Blanket mire+wet & dry heath	0.73		5
16	Moorhouse	Blanket mire	0.44		2.2
17	Fenns' Moss B	Raised bog	1.87		2.2
18	Stanford	Grassland and heathland	1.54		2.8
19	Fressingfield	Unimproved neutral grassland	5.29		3.7
20	Bedlington/Bedingfield	Unimproved neutral grassland	7.43		3.8
21	Borrowdale	Upland oak woodland	0.19		2
22	Brown Moss	Woodland and heathland	3.95		2.7
23	Llnclys Common	Grassland, scrub and woodland	1.78		1.8
24	Wytham Wood	Wood pasture	1.13		3.6
25	Lullington Heath	Chalk heath and grassland	0.9		3.3
26	Lough Navar	Oak woodland and blanket bog	0.47	0.36	0.6
27	Glenmore Wood	Mature acid oak woodland	2.28		0.7
28	Caldanagh Bog	Lowland raised bog	2.58		1.5
29	Castle Enigan	Lowland Fen	4.09		2
30	Orritor	Oak woodland	6.45		3.9
31	Redgrave and Lopham	Fenland	2.28		3.7
32	Yarner Wood	Ancient oak woodland	0.65	1.05	1.1

The N and S deposition values were derived from current CEH deposition maps and show a range in the sites from 5-73 kg N ha^{-1} y^{-1} and 7-28 kg S ha^{-1} y^{-1}. The N deposition is determined from either woodland or grassland estimates of modelled deposition maps, which are based on measured concentrations. Deposition for sites with predominately woodland habits have been calculated using deposition velocities for woodland and grassland sites using a grassland/moorland deposition velocity (see Sections 10, 11, 12, 13, 14 and 15).

These data will be discussed in context to their impacts on the lichen diversity, bark pH and moss N status.

Table 9.4. Total N and S deposition for the 32 UK sites. These deposition have been adjusted by habitat type for each specific site.

Site No.	Site Name	Total N deposition kg N ha^{-1} y^{-1}	Total S deposition kg S ha^{-1} y^{-1}
1	Cwmystwyth	30.3	12.1
2	Plynlimon	13.6	13.3
3	Dyffrn Mymbyr	31.3	13
4	Stackpole	28.4	12
5	Eskdalemuir	14.2	10.4
6	Halladale	11.1	11.0
7	Strathvaich Dam	7.9	14.2
8	Glensaugh	12.4	7.7
9	Inverpolly/Knockan	5.4	9.6
10	Edinburgh Centre	28.5	21.9
11	Ariundle	11.3	22.2
12	Glen Nant	17.7	22.9
13	Wood of Cree	16.2	12.8
14	Bush	16.6	11.2
15	Sherwood (Ladybower)	31.2	24.6
16	Moorhouse	20.1	12.8
17	Fenns' Moss B	21.8	7.4
18	Stanford	22.3	9
19	Fressingfield	40.9	9.3
20	Bedlington/Bedingfield	53.4	9.4
21	Borrowdale	40.1	28.6
22	Brown Moss	32.8	8
23	Llnclys Common	20.5	7.4
24	Wytham Wood	33.1	9.1
25	Lullington Heath	14.8	10.0
26	Lough Navar	7.0	14.9
27	Glenmore Wood	32.5	13.8
28	Caldanagh Bog	22.1	8.3
29	Castle Enigan	32.7	10.1
30	Orritor	73.0	10
31	Redgrave and Lopham	25.2	9.1
32	Yarner Wood	31.2	18.2

9.6. Sampling protocols

The sampling protocols for the pleurocarpous mosses were specifically designed for this project. The sampling procedures are detailed below.

PROTOCOL FOR COLLECTING MOSS SAMPLES FOR N BIOMONITORING PROJECT.

- Select three areas that are representative of the moss communities at your site.
- Ideally, the sampling area should not be greater than 50 x 50 m.
- If there is a current or has been a air pollution monitoring station, try to sample as close to this as possible. Record the distance (m) from the moss sampling area to the monitoring station on the data sheet provided.
- In areas with trees, each sampling point should be at least 3 m away from the base of the tree to avoid canopy drip from the overhanging branches.
- In open heathland, grassland or peatland areas, sampling below a canopy of shrubs and large-leafed herbs should be avoided, as well as in areas with running water on slopes.
- From these areas, select two of the commonest/most abundant species (see below for species types) and identify them if possible using a field guide. Moss samples should be collected from the ground and not tree trunks and branches wherever possible.
- If possible try to select species (see species list below) that are quick to collect and clean of soil, leaf litter and other plant material.
- Collection should if possible be when the moss surfaces are relatively dry but try to avoid collection during lengthy dry or wet periods.
- Using disposable gloves supplied, fill a (19 x 12.8 cm) plastic bag per species at each of the 3 areas i.e. 3 different areas x 2 species = 6 bags in total. Bags and disposable gloves will be supplied as part of pack.
- The samples collected should be live healthy tissue (green).
- Label (with the waterproof marker provided) all individual bags with:
    - Site name (moss sampling)
    - Date of collection
    - Name of moss (if possible)
    - Area number & name
    - Name of field officer

Mark the collections areas on a map (if one is available)

Please complete the site information sheet and the moss sampling data sheet.

Put the sample bags in the box provided and send it immediately (together with the map) by 9 July 2004 to:

Centre for Ecology and Hydrology Edinburgh

Bush Estate

Penicuik

Midlothian

EH26 0QB

Please store in a cool, shaded place if posting is delayed for any reason

The site operators were provided with a preferred species list (Table 9.5). They were also provided with sampling sheets (see below), which asked for details of the site in general, each of the sampling sites and the species of moss sampled. This form was returned with the sampled mosses.

It is recognised that there is seasonal variation in the foliar N content of mosses, with spring and autumn having the largest growth and nutrient uptake (Sutton *et al.* 2004a). This seasonality effect may also influence soluble NH_4-N concentrations, but this will not be established until concentrations are measured in the field throughout a year.

However, the timescale of the project meant that mosses had to be collected in early summer. To compensate for any variation due to weather conditions operators wee instructed to only sample when mosses were neither saturated nor very dry. The aim was to collect moss samples, which were as representative as possible of the on-site mosses.

Table 9.5. Preference list of pleurocarpous moss species for collection as part of the extensive UK study.

POSSIBLE SPECIES LIST IN ORDER OF PREFERENCE
1 *Rhytidiadelphus triquetrus*
2 *Rhytidiadelphus squarrosus*
3 *Rhytidiadelphus loreus*
4 *Pleurozium schreberi*
5 *Scleropodium purum*
6 *Hylocomium splendens*
7 *Eurhynchium praelongum*
8 *Thuidium tamariscinum*
9 *Hypnum cupressiforme*

For Lichen sampling protocols see Appendix III.

Moss Sampling Data Sheet

Site name			
JNCC Biomonitoring Site no.			
Site name (Conservation site, if applicable)			
Name of field officer			
	Area 1	**Area 2**	**Area 3**
Name of location (Area)			
Date of collection			
Brief description of location **(Distance of moss sampling area from air monitoring point, if applicable)**			
Name of Moss species 1			
Name of Moss species 2			
Comments			

10. Extensive scale UK study: tissue N content and soluble NH_4-N concentration in pleurocarpous mosses

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10.1. Introduction

This chapter reports the results of the pleurocarpous moss tissue N content and soluble NH_4-N concentration measurements, which were part of the extensive UK study. The sampling protocols and the UK site information are detailed in Section 9, whilst the biochemical analysis procedures for determining tissue N and soluble NH4-N are set out in Appendices I and II. Total N content and soluble NH_4-N concentration methods were tested in parallel with the lichen diversity methods.

10.2. Results

10.2.1. Pleurocarpous moss sampling

A total of 15 different moss species were returned by the UK conservation and environmental agencies field officers. The most frequently sampled moss was *Rhytidiadelphus squarrosus* followed by *Scleropodium purum* (Figure 10.1*).* These two species, in addition to being very common are probably both amongst the most recognisable of the species suggested for sampling. The moss samples returned to CEH were all unsorted; therefore CEH staff sorted the samples to confirm species identification and then cleaned them in preparation for chemical analysis. Full details of moss sample preparation are given in Section 9.

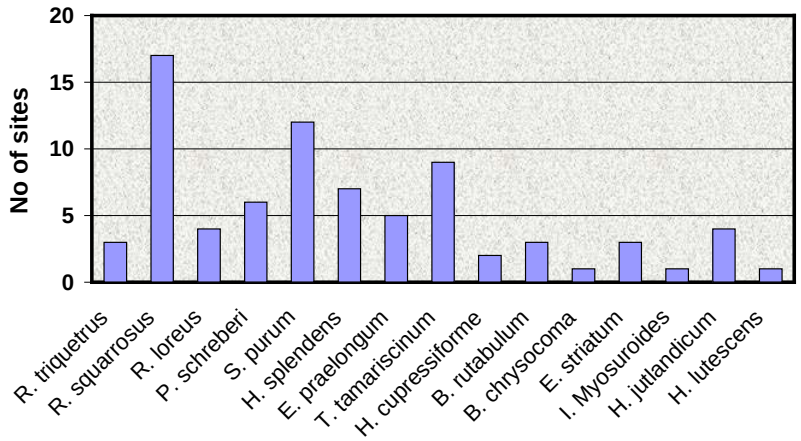

Figure 10.1. The species of pleurocarpous mosses sampled and the frequency of sampling for the individual species by the UK site operators.

Although, the main purpose of the moss assessment was to evaluate the chemical nitrogen indicators, it is also relevant to compare the species sampled with the different Ellenberg indicator scores for the individual species.

For bryophytes, N scores were obtained from Siebel (1993). In calculating the mean N index, an N score is assigned to each species on a scale 1-10. Table 10.1 illustrates the range of N scores for the moss species collected in the UK extensive study. The mosses collected in the UK extensive survey had N scores ranging from 2-8. *Brachythecium rutabulum,* a woodland species had a high score of 8 whereas, *Pleurozium schreberi* and *Hylocomium splendens,* open moorland species had scores of 2. This wide range of Ellenberg scores in the moss species obtained helps explain why so many species ended up being included in the assessment.

At the outset, given the clearly stated priorities for species sampling (described in full within Section 9.6), it was expected that the preferred species would be sampled to a higher degree and the other species less often. It is notable that the field officers also sampled several other species not included in the preference list. This may indicate a requirement to select fewer priority species in the guidance and also to stress the importance of only sampling those species identified in the preferred list. In some cases, non-priority species were clearly sampled instead of priority species, even though the habitats sampled would indicate the priority species would be present.

Table 10.1. The Ellenberg index N values for the 15 mosses sampled in the UK study.

Species	Ellenberg Index
Brachythecium rutabulum	8
Breutelia chrysocoma	-
Eurhynchium praelongum	6
Eurhynchium striatum	5
Homalothecium lutescens	-
Hylocomium splendens	2
Hypnum cupressiforme	3
Hypnum jutlandicum	-
Isothecium myosuroides	4
Pleurozium schreberi	2
Rhytidiadelphus squarrosus	5
Rhytidiadelphus loreus	2
Rhytidiadelphus triquetrus	3
Scleropodium purum	5
Thuidium tamariscinum	4

Tissue N content is amongst the most studied of the bioindicators methods, whereas, the use of soluble NH_4-N concentration has only recently been tested in the field (Sutton *et al.* 2004).

One of the objectives of the current study was to refine and test the suitability of the soluble NH_4-N concentration method under different field conditions, using a range of moss species as a bioindicator of N deposition. All moss species collected in the intensive and UK extensive study were analysed for both total N content (% dry weight) and soluble NH_4-N concentration.

The relationship between tissue N content (% dry weight) and soluble NH_4-N is shown in Figure 10.2. This graph includes all the extensive and intensive site moss data and also the *Deschampsia flexuosa* data (N=360). There appears to be a reasonably good relationship (R^2 =0.6175) between the two methods especially considering it includes grass biomonitors and mosses from a variety of habitats. Figure 10.2 shows that the response function for soluble foliar NH_4-N concentration is approximately 20 times greater than N content, which is consistent with the results of previous studies conducted adjacent to a poultry farm in Scotland (Sutton *et al*. 2004a).

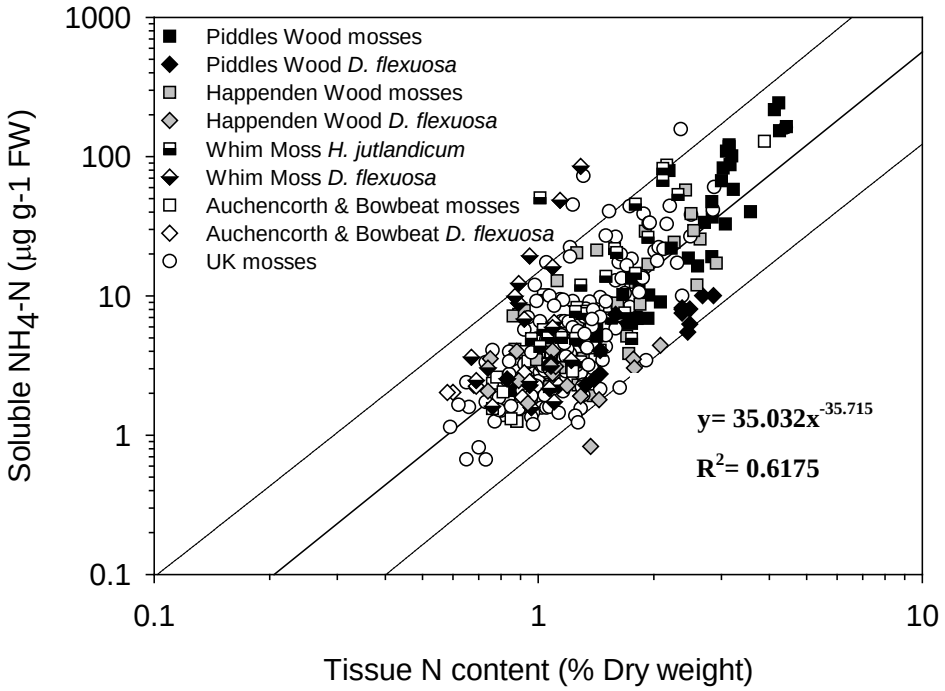

Figure 10.2. Relationship between tissue N content and soluble NH_4-N concentration for pleurocarpous mosses from intensive and extensive sites across the UK and *Deschampsia flexuosa* from the intensive sites, with 95% confidence intervals.

The relationship between tissue N content and soluble NH_4-N for pleurocarpous mosses only (Figure 10.3), collected from 32 extensive sites throughout the UK, is not as strong (R^2=0.554) when compared against both moss and grass bioindicators (as shown in Figure 10.2). However, when individual species (*R. squarrosus, T tamariscinum Eurynchium* spp.*)* are compared there are much stronger relationships found (Table 10.2). *S. purum* is the exception, with little variation (R^2= 0.505) from the R^2= 0.48 found for all UK mosses.

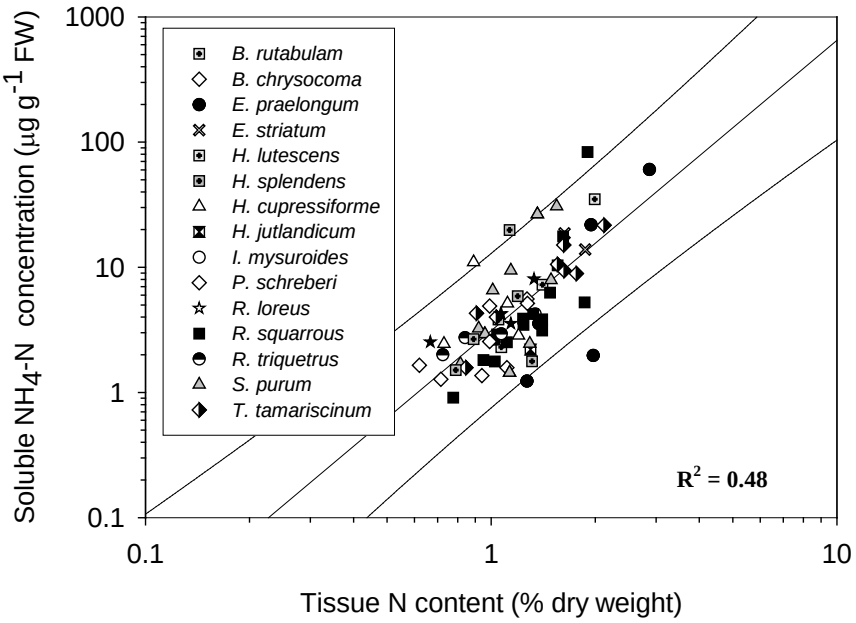

Figure 10.3. The relationship between tissue N content and soluble NH_4-N concentration for pleurocarpous mosses from extensive sites in the UK study.

There was a significant but scattered relationship between tissue N content and atmospheric NH_3 concentration for all moss species in the UK study (Figure 10.4; R^2 =0.365, p= 0.03). A similar response was also found for UK sites, between soluble NH_4-N concentrations and NH_3 concentrations (Figure 10.5) R^2= 0.277, p=0.01. This variability could be the attributed to patterns of N exposure (i.e. different ratios of wet and dry N deposition rates) and/or habitat variation for the individual mosses species. Additionally, the range of total N content was quite large (0.62% - 2.87%) and especially at two sites (Fressingfield, Orritor) where the foliar N content in the sampled mosses was over 2% N content.

There was also a considerable range of NH_3 concentrations (0.04-7.4 μg m^{-3}) across the 32 selected UK-wide extensive sites. Although differences in foliar N contents found in this study can be related to wet and dry N inputs, it is important to recognise that both intra and inter-species differences in moss nitrogen levels occur (Pitcairn *et al*., 2002). Sutton *et al.* (2004) found that the N content differences between *P. schreberi and R. squarrosus* sampled from a 'clean' blanket bog were 0.75-0.85% and 0.88-1.37% respectively. In this current study, differences in N content between *Pleurozium schreberi* and *Rhytidiadelphus squarrosus* ranged from 0.62-1.27% N and 0.78-1.90% N respectively.

Table 10.2 summaries the results from the UK extensive study. Data includes relationships between all the UK moss species and selected individual moss species where there were a sufficient number of sampling sites. The measured NH_3 concentrations and total estimated N deposition are also listed.

Table 10.2. Summary of correlations for all UK mosses and individual moss species.

		NH_4^-N concentration µg g^{-1} FW	**N deposition kg N ha^{-1} y^{-1}**	**NH_3 concentration µg m^{-3}**
% N (all spp. moss)	R^2	0.480	0.262	0.365
	N	75	75	75
	p=	<0.001	<0.001	0.034
NH_4-N (all spp. moss)	R^2	-	0.232	0.278
	N	-	75	75
	P=	-	<0.001	<0.001
% N (*R. squarrosus*)	R^2	0.354	0.438	0.347
	N	16	16	16
	p=	0.108	<0.001	<0.001
NH_4-N (*R. squarrosus*)	R^2	-	0.384	0.521
	N	-	16	16
	p=	-	<0.001	0.140
% N (*S. purum*)	R^2	0.505	0.348	0.033
	N	13	13	13
	p=	0.0104	<0.001	0.229
NH_4-N (*S purum*)	R^2	-	0.267	0.054
	N	-	13	13
	p=	-	<0.001	0.018
% N (*T. tamariscinum*)	R^2	0.823	0.260	0.229
	N	8	8	8
	p=	0.003	0.003	0.637
NH_4-N (*T tamariscinum*)	R^2	-	0.522	0.468
	N	-	8	8
	p=	-	0.050	0.008
% N (*Eurynchium praelongum* and *Eurynchium striatum*)	R^2	0.7788	0.6865	0.362
	N	7	7	7
	p=	0.071	<0.001	0.006
NH_4-N (*Eurynchium praelongum* and *Eurynchium striatum*)	R^2	-	0.575	0.177
	N	-	7	7
	p=	-	0.079	0.010

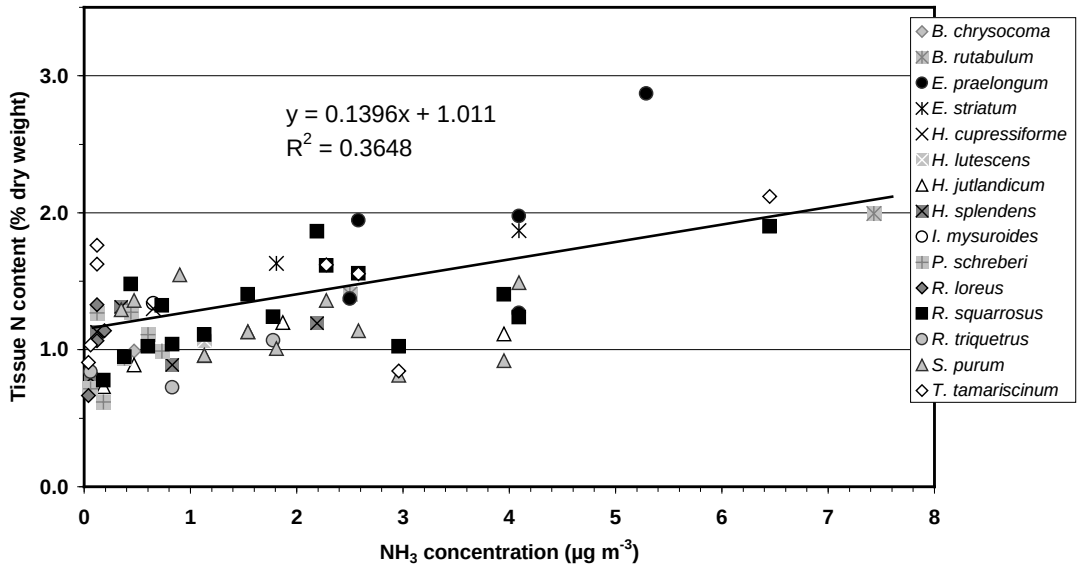

Figure 10.4. Effects of NH_3 concentration on tissue N content for pleurocarpous mosses sampled in the UK study.

In both Figures 10.4 and 10.5 specific species appear to have the highest foliar N content and soluble NH_4-N concentrations and also appear at the sites with the highest ambient NH_3 concentrations (*B. rutabulum, T. tamariscinum* and *E. praelongum*).

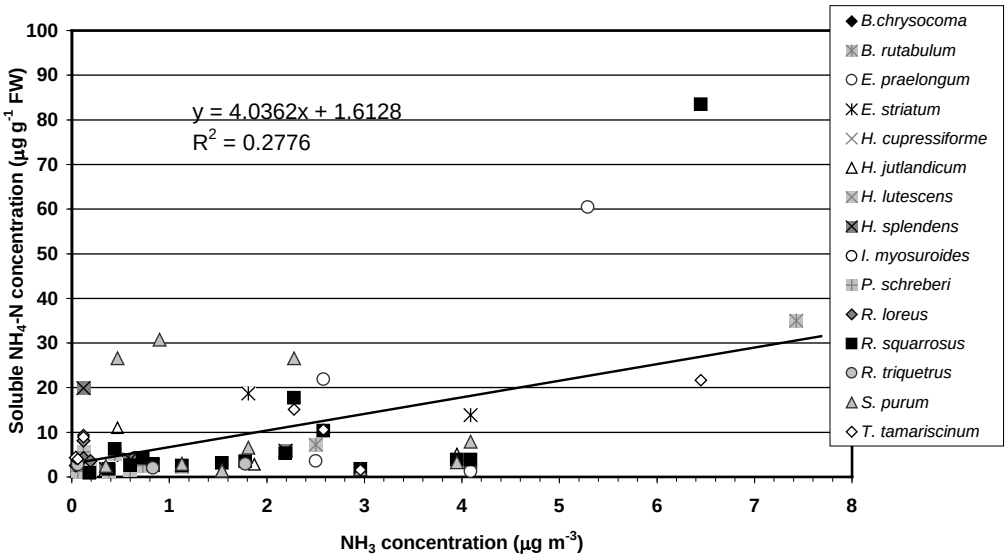

Figure 10.5. Relationship between measured NH_3 concentration and soluble NH_4-N concentration for pleurocarpous mosses sampled in the UK extensive study.

There was a weak relationship found for all tissue N content and NH_4-N concentrations in the UK mosses and estimated N deposition (Figures 10.6 and 10.7). The relationship for tissue N content and N deposition is weaker than that found for tissue N content and NH_3 concentration (Figure 10.4). There is virtually no difference in the soluble NH_4-N relationship between NH_3 concentration and/or N deposition (R^2 = 0.232 and 0.277 respectively).

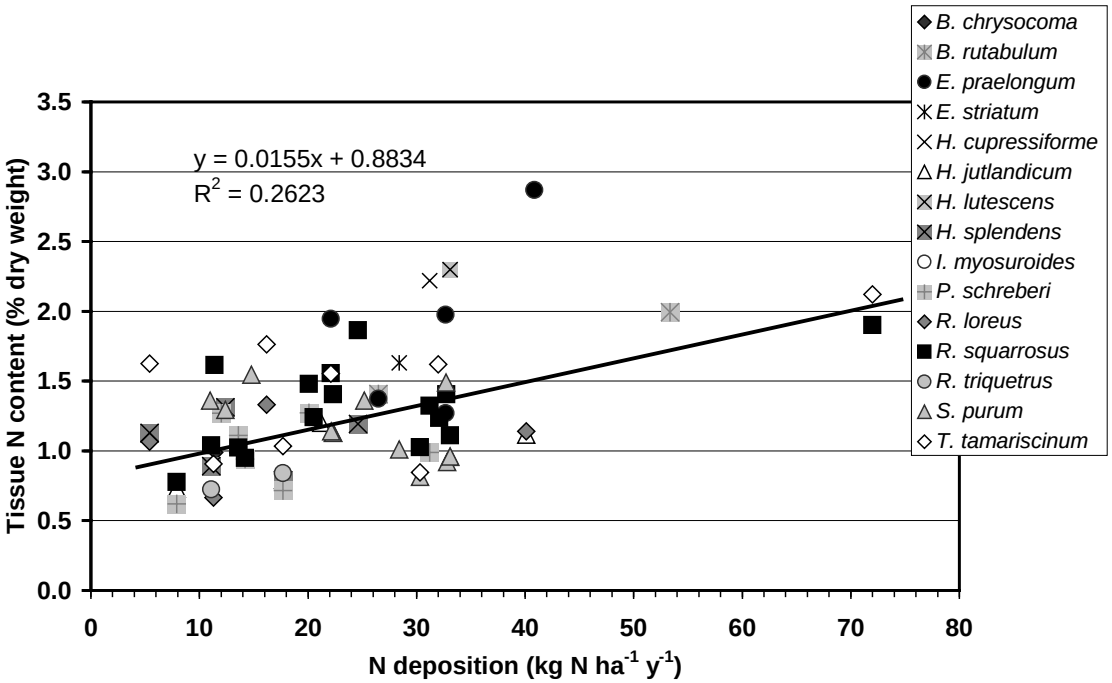

Figure 10.6. Effects of total N deposition on tissue content for all the pleurocarpous mosses sampled in the UK study.

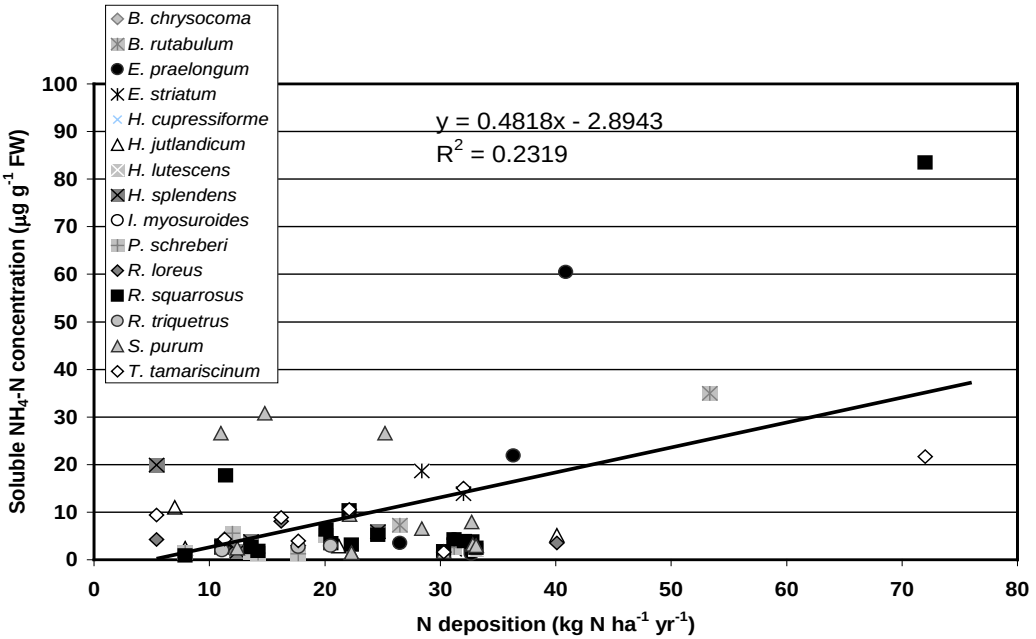

Figure 10.7. Effects of total N deposition on soluble NH_4-N concentration for pleurocarpous mosses sampled in the UK study.

Figures 10.4 to 10.7 use all the available moss species data from the UK extensive study. However, it was found that by removing the *Scleropodium purum* and *Thuidium tamariscinum* data a much better relationship could be achieved for both tissue N content and NH_4-N concentration against atmospheric UK NH_3 concentrations as experienced at individual sites (Figure 10.8). The relationship between both total N content and soluble NH_4-N concentration in *S. purum* and atmospheric NH_3 concentration is particularly poor R^2=0.033 and R^2=0.054 respectively (Table 10.2).

However, removing *Scleropodium purum* and *Thuidium tamariscinum* does not improve the relationship between tissue N content and NH_4-N concentration against N deposition (R^2=0.274 and R^2=0.275 respectively for *S. purum and T. tamariscinum*), indicating that they have a better relationship with N deposition than dry NH_3 concentration.

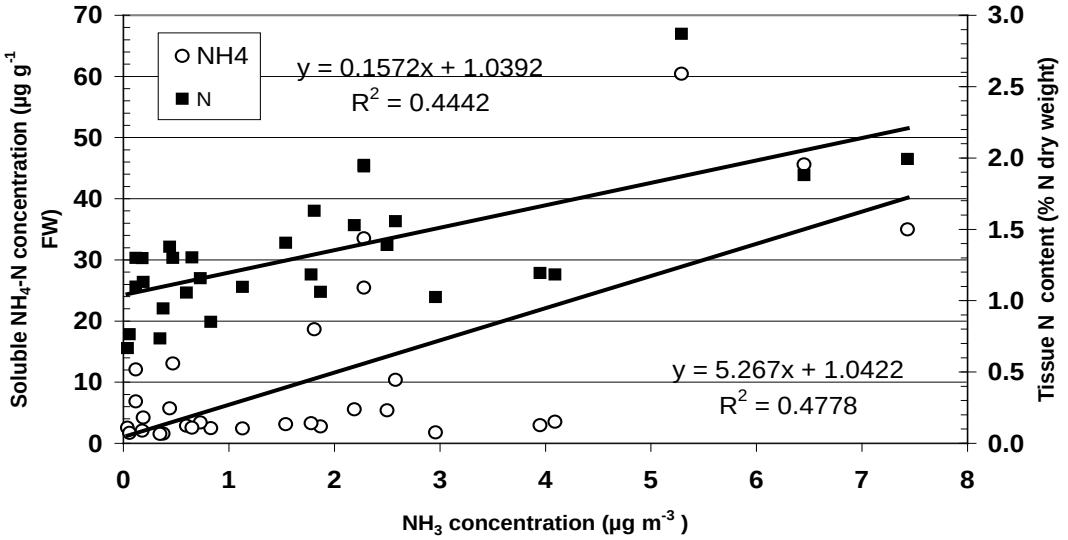

Figure 10.8. The relationship between UK moss data and ammonia concentration without the *Scleropodium purum* and *Thuidium tamariscinium* data.

There is also evidence that the interaction between N deposition and precipitation rates affect moss foliar chemistry. While the correlation between total tissue N and N deposition for the UK samples (excluding *Scleropodium purum* and *Thuidium tamariscinium*) is R^2 =0.437 (Figure 10.8), the correlation including both precipitation and N deposition increases to R^2= 0.524 for the same moss species (Figure 10.9). The precipitation data used were the 5 year annual mean precipitation volumes (1999-2003) for the 5 x 5 km grid square in which the individual UK extensive site was located. There was a correction factor of 0.041 added to the precipitation data.

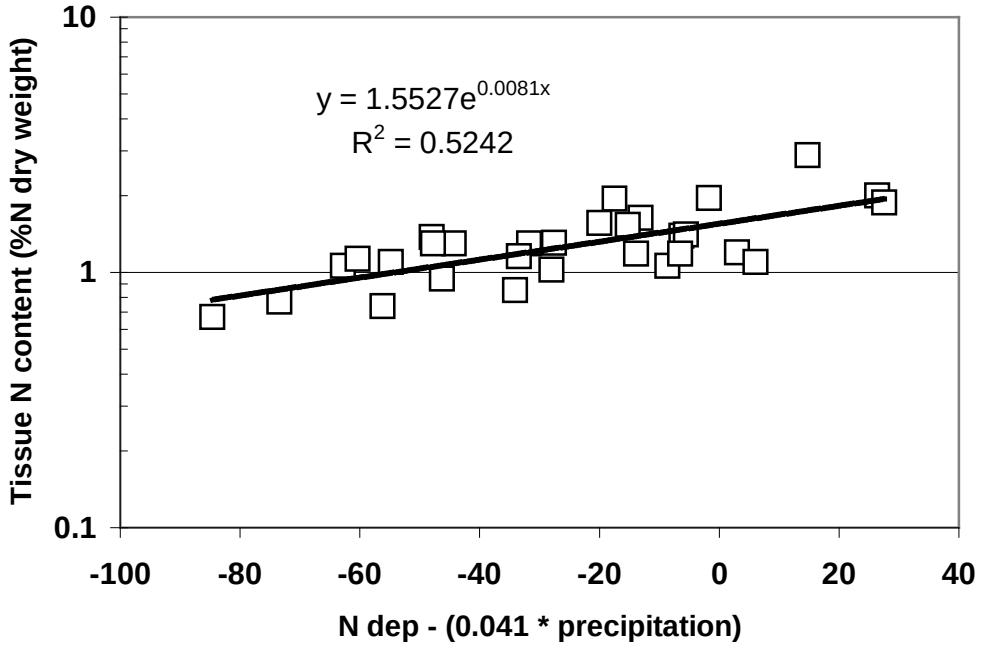

Figure 10.9. Multiple regression of UK moss tissue N content data against N deposition (kg N ha^{-1} y^{-1}) and annual precipitation (mm). Inclusion of the precipitation interaction increases R^2 from 0.437 to 0.524. The data presented are plotted excluding *Scleropodium purum* and *Thuidium tamariscinium*.

Plotting the most frequently sampled pleurocarpous moss in the UK extensive sites, *R. squarrosus* against NH_3 concentration shows a reasonable linear relationship (R^2=0.512) with soluble NH_4-N concentration (Figure 10.10). There was also a relationship with total N content but this was not as strong (R^2=0.347). The relationship for total N content in *R. squarrosus* improves slightly when plotted against N deposition but conversely the NH_4-N concentration relationship is poorer (Figure 10.11). It is thought that sites differences with variations in climate, moisture, shade, vegetation exposure, management practices and grazing pressures may be contributing factors in the moss nutrient values as well as wet and dry N inputs.

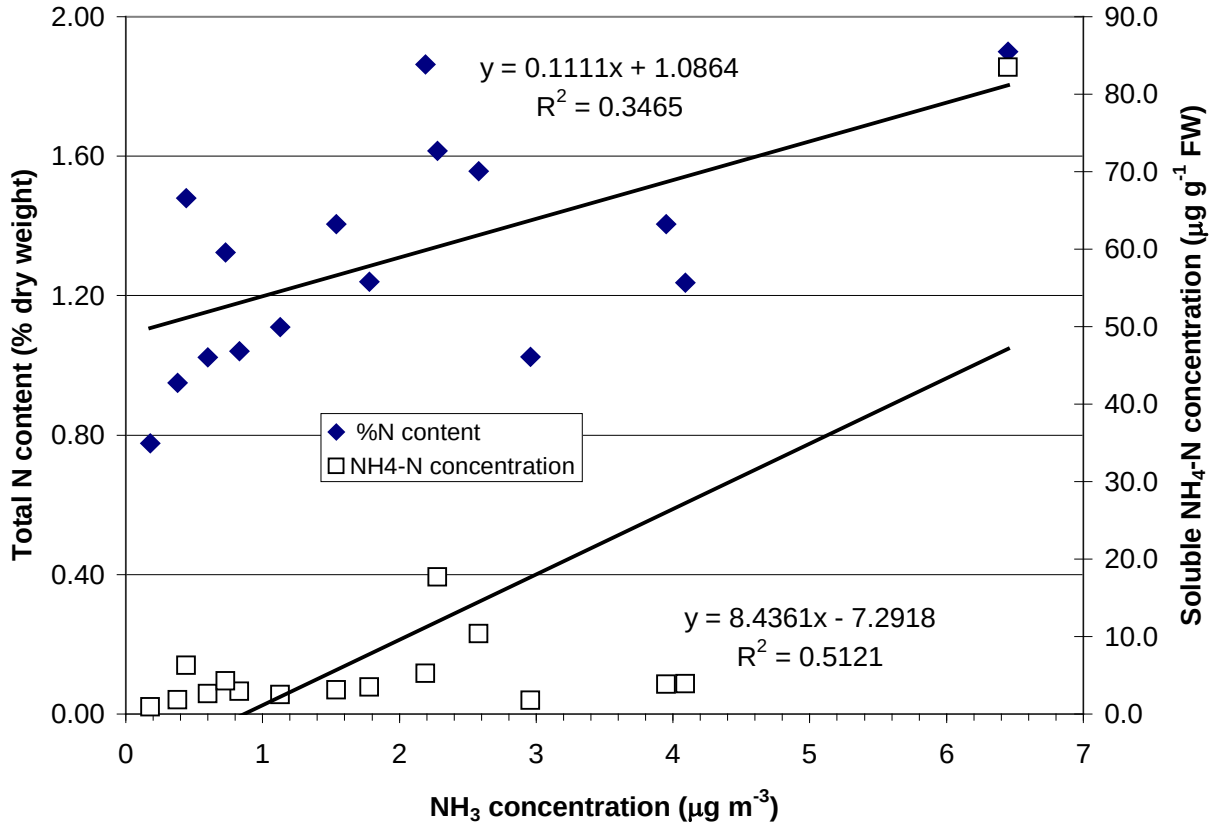

Figure 10.10. Relationship between total N content and soluble NH_4-N concentration and NH_3 concentration for *R. squarrosus* sampled in the UK extensive study.

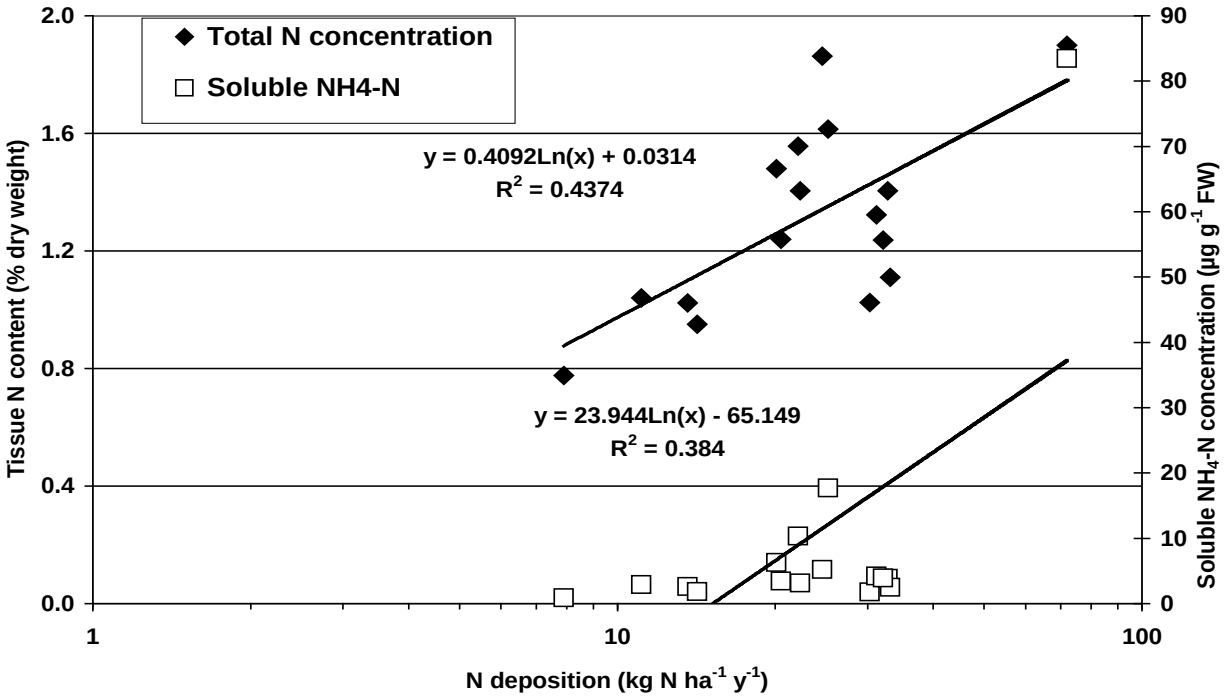

Figure 10.11. Effects of log total N deposition on total N content and soluble NH_4-N concentration for *R. squarrosus* sampled in the UK extensive study.

Assessing the relationship between other individual moss species (*S. purum* and *T. tamariscinium* and *Eurynchium* spp.) and NH_3 concentration against N deposition exhibited a strong interspecies variation amongst mosses. *S. purum* as previously reported had the poorest relationship between N content and NH_4-N concentrations against atmospheric NH_3 concentrations R^2= 0.033 and R^2=0.054 respectively when compared against the other mosses sampled (Table 10.2).

There was little difference in the *T. tamariscinium* Foliar N content and NH_4-N relationships with N deposition and NH_3 concentration (Table 10.2). However, the *Eurynchium* spp. relationship is much stronger with N deposition than NH_3 concentration.

These data, including the results for *R. squarrosus* have identified moss species and their specific relationship with either wet or dry N deposition, which could be used to target specific species to specific atmospheric N inputs.

10.3. Comparison of the use of critical load as an indicator of monitoring or integrity compared to biomontor methods applied in the current study

Currently the conservation agencies and environment agencies use critical loads to help assess the impacts or potential impacts on the integrity of designated sites across the UK. The definition of a critical load is a 'quantative estimate of exposure to one or more pollutants below which significant harmful effects on sensitive elements of the do not occur according to present knowledge' (Nilsson and Greenfelt 1988). The critical load approach is dependent on the accuracy of N deposition estimates and the critical value set for each of the designated habitat types. These have recently been reviewed at a workshop on empirical critical loads for N deposition (Achermann and Bobbink 2003). The critical load for individual habitats is determined by a combination of results from experimental studies and expert judgement, with the degree of certainty for individual habitat types varying depending on the current data available. The reliability of the critical loads set for individual habitats ranges from reliable for raised bogs and mires to being based on expert judgement only for lichens and algae in temperate and boreal forests (Achermann and Bobbink, 2003). Therefore, when considering the use of critical loads for a specific habitat it is important to determine the reliability of the critical load for that habitat.

The 32 sites selected for the UK extensive study are from a range of habitat types and atmospheric N inputs with N deposition ranging from 5.4 kg N ha^{-1} yr^{-1} in wet N dominated NW Scotland (Site 9, Knockan, Inverpolly) to 73 kg N ha^{-1} y^{-1} for Site 30, Orritor, Northern Ireland:.

Using the CEH estimates of N deposition to these 32 sites (See Section 3), incorporating the results from air monitoring where available (Table 10.3), three out of the four Welsh sites, all thirteen of the English sites and all Northern Ireland sites, except for Lough Navar exceeded the critical load for their designated habitat types.

In Scotland, only two parkland sites (Edinburgh Centre and Bush Estate) exceed the critical load, although Glen Nant and Wood of Cree are marginal. Therefore, using the critical load alone it can be assumed that these sites are currently at risk or have potential for change in integrity.

One of the aims of the current study was to assess if the application of one or a combination of the N biomonitoring methods would be an important addition to the CSM or be a useful tool for individual sites where a potential N effect has been identified. Therefore, when the values of the moss foliar chemical indicators are compared with critical loads it can be seen that they do reflect the N input especially the dry N deposition (Table 10.4 and Section 10.4).

Table 10.3. Sites used in the UK extensive study showing NH_3 concentration, N deposition, Critical load exceedance for the individual habitats, with current Critical Load exceedance indicated for the relevant sites.

Site no.	Site	NH_3 concentration $\mu g\ m^{-3}$	N deposition kg N ha^{-1} y^{-1}	Critical Load kg N ha^{-1} y^{-1}	Exceedance of CL
1	Cwmystwyth	2.96	30.3	10 - 20	✓
2	Plynlimon	0.6	13.6	10 -20	
3	Dyffrn Mymbyr	1.18	31.3	10 - 20	✓
4	Stackpole	1.81	28.4	10 -15	✓
5	Eskdalemuir	0.38	14.2	10 - 20	
6	Halladale	0.83	11.1	10 - 20	
7	Strathvaich Dam	0.18	7.9	10 - 20	
8	Glensaugh	0.35	12.4	10 - 20	
9	Inverpolly/Knockan	0.12	5.4	10 -20	
10	Edinburgh Centre	2.5	26.5	10 -20	✓
11	Ariundle	0.04	11.3	10 - 15	
12	Glen Nant	0.06	17.7	10 - 15	✓
13	Wood of Cree	0.12	16.2	10 - 15	✓
14	Bush	0.9	16.6	10 - 15	✓
15	Sherwood (Ladybower)	0.73	31.2	5 - 10	✓
16	Moorhouse	0.44	20.1	5 - 10	✓
17	Fenns' Moss B	1.87	21.2	5 - 10	✓
18	Stanford	1.54	22.3	10 - 20	✓
19	Fressingfield	5.29	40.9	20 - 30	✓
20	Bedlington/Bedingfield	7.43	53.4	20 -30	✓
21	Borrowdale	0.19	40.1	10 - 15	✓
22	Brown Moss	3.95	32.8	10 - 20	✓
23	Llnclys Common	1.78	20.5	10 - 20	✓
24	Wytham Wood	1.13	33.1	10 - 15	✓
25	Lullington Heath	0.9	15.0	15 - 25	✓
26	Lough Navar	0.47	11.5	10 - 15	
27	Glenmore Wood	2.28	32.5	10 - 15	✓
28	Caldanagh Bog	2.58	22.1	10 - 15	✓
29	Castle Enigan	4.09	32.7	10 - 20	✓
30	Orritor	6.45	72.9	10 - 20	✓
31	Redgrave and Lopham	2.28	25.2	15 - 20	✓
32	Yarner Wood	0.65	31.2	10 - 20	✓

10.4. Determination of N impact using a threshold tissue N content value

Using expert judgement based on previous studies looking at foliar N accumulation of moss species (Pitcairn *et al.* 1995, 1998) a value of 1.3% N was assigned as the threshold foliar N content value for pleurocarpous moss species. Therefore, concentrations > 1.3% N were taken as an indication of a) elevated N concentrations due to N deposition and b) potential N impacts on the designated site. Using the total N content (% N dry weight) for the moss samples collected as part of the UK extensive study, Table 10.4 lists those sites with mosses exhibiting % N contents >1.3% N, whilst Table 10.5 lists those sites with a tissue N content threshold value of < 1.3% N.

Table 10.4. Sites with pleurocarpous moss sampling exhibiting foliar %N values > 1.3% N threshold content.

Site number	Location	Species	NH_3 conc. $\mu g\ m^{-3}$	N dep. (kg N ha^{-1} y^{-1})	NH_4-N conc. ($\mu g\ g^{-1}$ FW)	Tissue N content (% DW)
4	Stackpole	*Eurhynchium striatum*	1.81	28.4	18.66	1.63
8	Glensaugh	*Hylocomium splendens*	0.35	12.4	1.77	1.31
9	Inverpolly/Knockan	*Thuidium tamariscinum*	0.12	5.4	9.41	1.63
10	Edinburgh Centre	*Eurhynchium praelongum*	2.5	26.5	3.55	1.37
10		*Brachythecium rutabulam*			7.26	1.41
13	Wood of Cree	*Rhytidiadelphus loreus*	0.12	16.2	8.08	1.33
13		*Thuidium tamariscinum*			8.92	1.76
14	Bush	*Rhytidiadelphus squarrosus*	0.90	16.6	5.25	1.86
15	Sherwood (Ladybower)	*Rhytidiadelphus squarrosus*	0.73	31.2	4.24	1.32
16	Moorhouse	*Rhytidiadelphus squarrosus*	0.44	20.1	6.28	1.48
18	Stanford	*Rhytidiadelphus squarrosus*	1.87	22.3	3.13	1.41
19	Fressingfield	*Scleropodium purum*	5.29	40.9	7.25	1.36
19		*Eurhynchium praelongum*			60.44	2.87
20	Bedlington/ Bedingfield?	*Brachythecium rutabulam*	7.43	53.4	34.96	1.99
22	Brown Moss	*Rhytidiadelphus squarrosus*	3.95	32.8	3.84	1.41
25	Lullington Heath	*Scleropodium purum*	0.9	14.8	30.78	1.55
26	Lough Navar	*Scleropodium purum*	0.47	11.5	26.61	1.36
26		*Rhytidiadelphus squarrosus*			17.69	1.62
27	Glenmore Wood	*Eurhynchium striatum*	2.28	32.5	13.78	1.87
27		*Eurhynchium praelongum*			31.3	1.98
7		*Thuidium tamariscinum*			15.08	1.62
28	Caldnagh Bog	*Rhytidiadelphus squarrosus*	2.58	22.1	10.36	1.56
28		*Thuidium tamariscinum*			10.56	1.56
29	Castle Enigan	*Thuidium tamariscinum*	4.09	32.7	12.91	1.59
29		*Scleropodium purum*			7.92	1.49
30	Orritor	*Thuidium tamariscinum*	6.45	72.9	21.69	2.12
30		*Eurhynchium praelongum*			21.89	1.95
30		*Rhytidiadelphus squarrosus*			83.49	1.90
31	Redgrave &Lopham	*Brachythecium rutabulam*	2.28	25.2	33.51	1.95
32	Yarner Wood	*Hypnum cupressiforme*	0.65	31.2	2.22	1.30

Table 10.5. UK Sites with moss with a foliar N content threshold of <1.3% N.

Site No.	Location	Species	NH_3 conc. $\mu g\ m^{-3}$	N dep. (kg N ha^{-1} y^{-1})	NH_4-N conc. ($\mu g\ g^{-1}$ FW)	Tissue N (% DW)
1	Cwmystwyth	*Scleropodium purum*	2.96	30.3	1.70	0.82
1		*Rhytidiadelphus squarrosus*			1.77	1.03
1		*Thuidium tamariscinum*			1.58	0.85
2	Plynlimon	*Pleurozium schreberi*	0.6	13.6	1.58	1.05
2		*Hylocomium splendens*			3.84	1.11
2		*Rhytidiadelphus squarrosus*			2.64	1.02
4	Stackpole	*Scleropodium purum*	1.81	28.4	6.57	1.01
5	Eskdalemuir	*Pleurozium schreberi*	0.38	14.2	1.37	0.94
5		*Rhytidiadelphus squarrosus*			1.82	0.95
6	Halladale	*Rhytidiadelphus triquetrus*	0.93	11.1	2.00	0.73
6		*Hylocomium splendens*			2.67	0.89
6		*Rhytidiadelphus squarrosus*			2.91	1.04
7	Strathvaich Dam	*Hypnum jutlandicum*	0.18	7.9	2.45	0.73
7		*Rhytidiadelphus squarrosus*			0.91	0.78
7		*Pleurozium schreberi*			1.65	0.62
8	Glensaugh	*Scleropodium purum*	0.35	12.4	2.46	1.29
9	Inverpolly/Knockan	*Rhytidiadelphus loreus*	0.121	5.4	4.25	1.07
9		*Hylocomium splendens*			19.88	1.13
11	Ariundle	*Rhytidiadelphus loreus*	0.04	11.3	2.54	0.67
11		*Thuidium tamariscinum*			4.30	0.91
12	Glen Nant	*Thuidium tamariscinum*	0.06	17.7	4.00	1.04
12		*Rhytidiadelphus triquetrus*			2.74	0.84
12		*Pleurozium schreberi*			1.28	0.72
12		*Hylocomium splendens*			1.51	0.79
13	Wood of Cree	*Pleurozium schreberi*	0.12	16.2	5.58	1.27
14	Bush	*Hylocomium splendens*	2.19	24.6	5.88	1.19
15	Sherwood (Ladybower)	*Pleurozium schreberi*	0.73	31.2	2.56	0.99
15		*Rhytidiadelphus squarrosus*			4.24	1.30
16	Moorhouse	*Pleurozium schreberi*	0.44	20.1	5.15	1.27
17	Fenns' Moss B	*Hypnum jutlandicum*	1.87	21.2	2.86	1.20
17		*Scleropodium purum*			3.25	0.92
18	Stanford	*Scleropodium purum*	1.54	22.3	1.44	1.13
21		*Hypnum jutlandicum*	0.19	40.1	5.15	1.12
21	Borrowdale	*Thuidium tamariscinum*			4.83	1.06
22	Brown Moss	*Hypnum sp.*	3.95	32.8	1.25	0.77
22		*Scleropodium purum*			1.29	1.29
23	Llnclys Common	*Scleropodium purum*	1.78	20.5	2.94	1.08
23		*Rhytidiadelphus squarrosus*			3.48	1.24

23		*Rhytidiadelphus squarrosus*				1.29
24	Wytham Wood	*Scleropodium purum*	1.13	33.1	2.93	0.96
24		*Rhytidiadelphus squarrosus*			2.52	1.11
26	Lough Navar	*Breutelia chrysocoma*	0.47	11.5	4.91	0.99
26		*Hylocomium splendens*				0.98
28	Caldanagh Bog	*Scleropodium purum*	2.58	22.1	9.45	1.14
29	Castle Enigan	*Hypnum compressiforme*	4.09	32.7	2.40	1.02
29		*Rhytidiadelphus squarrosus*			3.89	1.24
32	Yarner Wood	*Eurhynchium praelongum*	0.65	31.2	1.23	1.27

It was discussed earlier in this section that there are species differences in foliar N content of mosses irrespective of local N deposition and that other factors such as microclimate, seasonality and habitat could play a part in influencing foliar N content. Accepting that species variations in N content exists, this section determines if a threshold foliar N value that could be assigned as a generalised indicator of N impacts irrespective of habitat and also to determine if there was a detectable relationship between sites with >1.3% N deposition data and critical load exceedance.

The results show that 20 out of the 32 UK extensive sites had pleurocarpous mosses with foliar tissue % N contents > 1.3% N thus indicating that current N deposition was impacting on these sites. Using the critical load data, 23 out of the 32 sites exceeded the critical load for their specific habitat. This shows that using threshold value agrees in general with the critical load exceedance values. The advantage of the threshold value over the critical load exceedance is that it assigns an actual value, which could be used as a scaler of N impact if sufficient data were available.

Despite the rather low correlations overall between the chemical nitrogen measurements of mosses and estimated nitrogen deposition or ammonia concentration, the site by site comparison from Tables 10.4 and 10.5 are very informative and show how the indicators overall pick out main sites where N deposition is known to be a problem. In particular, sites with high atmospheric NH_3 are identified, while sites with high wet deposition exceeding the critical load (such as Borrowdale and Yarner Wood) give lower values for the moss chemical indicators. The sites of key concern include; Bedlington, Glenmore Wood, Castle Enigan, Orritor and Redgrave & Lopham Fens. By contrast, the main mis-allocations using the moss bioindicator values would be Lough Navar and Lullington Heath, both of which have NH_3 and N deposition lower than critical thresholds. In part, this may be linked to the sampling of *S. purum* at these sites, although further explanation is needed to ascertain why the moss N indicators are so high at Lough Navar for *R. squarrosus.*

Similarly, it is surprising that Inverpolly/Knockan is included in Table 10.4 as exceeding the foliar chemical thresholds, in this case through recording of *T. tamariscinum*. Such effects point to the need for further detailed sampling of multiple moss species at each of these sites to establish whether the values recorded here were atypical or typical for the site. If the measurements at Inverpolly, Lough Navar and Lullington are atypical, this points to the need for more intensive sampling in future protocols. Conversely, if the values recorded are good representations of the mean site values, then more detailed analysis is required to understand the causes.

Finally, it should be noted that the present assessment was conducted by sampling at only one time of year, and further work is needed to characterise the seasonal variability of these chemical bioindicators.

10.5. Conclusions

- Overall, there is weak but significant relationship between N content and soluble NH_4-N versus NH_3 air concentration when comparing all mosses from the UK sites. There was also a significant and again weak relationship for the data as a whole with modelled nitrogen deposition. This indicates that moss N content and soluble NH_4-N are both responding to total wet and dry deposition. However, the slightly lower response of moss foliar N chemistry to wet than dry deposition is consistent with previous studies.

- The removal of *S. purum* and *T. tamariscinium* data improved the relationship between both % N content and soluble NH_4-N concentration and NH_3 concentration for the combined UK moss data but did not effect the relationship with N deposition.

- The lack of strong relationships with foliar concentrations and N inputs in the UK mosses could be due to site differences; with variations in climate, moisture, shade, vegetation exposure, management practices and grazing pressures. In particular, there are notable outliers in the overall relationships between N and foliar chemistry that should be further investigated. These include the results at Inverpolly, Lough Navar and Lullington Heath, which each have low N deposition and NH_3, but showed high foliar N and NH_4-N concentration. It needs to be clarified whether these results were the consequence of unrepresentative sampling at the site, or if the values are representative, why they are high at these sites.

- The inclusion of precipitation in a multiple regression with N deposition improves the relationship with tissue N content for all the UK moss data (excluding *S. purum* and *T. tamariscinium*). This indicates the importance of other factors in the relationship between N uptake by mosses and N inputs.

- Assessment of the relationships for different species, suggest that some species may be more suitable than others as bioindicators of atmospheric N inputs. In particular, while *Rhytiadelphus* species are suited to the assessment of both wet and dry N inputs, *Scleropodium purum* appears to be less well suited especially to NH_3 concentrations.

- The high degree of scatter in the relationships indicate that the N content and NH_4-N concentrations in mosses at sites with a diffuse N source, low N input (often wet N deposition dominated) may not produce a sufficient signal to allow this method to be used on its own predict site conditions reliably. These points to the need for increased replication and the use of foliar N chemistry in parallel with other biomonitoring methods.

- In relation to the sampling representativity, it may be hypothesized that the high values at Inverpolly, Lough Navar and Lullington Heath are due to non-representative sampling. If this is the case, the consequences point to the need to improve the guidance protocols to obtain representative sampling.

- By comparison with this UK wide assessment, the relationships of % N content and soluble NH_4-N concentration are much better for the intensive sites with a defined N point source (see Section 6).

- The UK sites selected were all diffuse N deposition sites, with approximately 44% dominated by dry N deposition and the 54% by wet N deposition. A site was assessed to be dry N dominated if > 50% of the total N was as NH_3-N deposition. As they were all diffuse sources this may account for the observed weak relationship with N deposition.

- There is a reasonable relationship between total N content and soluble NH_4-N concentration using all the UK site data (R^2= 0.48). This would indicate that either method could be used at conservation sites to give an early indication if N is impacting on the site. By contrast, the use of both methods in parallel has a significant advantage in improving the robustness of the results.

- *R. squarrosus* was the most frequently sampled moss in the UK Extensive study (17 out 32 sites). This moss is an easily recognised species and is abundant throughout the UK.

- There was a weak relationship between N content, soluble NH_4-N concentration in mosses and NH_3 concentration/ N deposition.

- Using a threshold value of 1.3% N as an indication of N impacts showed that 20 out of the 32 sites were being currently impacted upon by N deposition. This could have long- term problems for the integrity of these sites. Using the critical loads criteria demonstrated that 23 out of the 32 had N deposition above the current load for their specific habitat, whereas the threshold % N data indicated that 20 could be being impacted upon. The advantage of the bioindicator threshold value over the critical load exceedance is that it assigns an actual value, which could be used as a scaler of N impact if sufficient data was available.

- The combination of foliar % N content and soluble NH_4-N in mosses successfully identified the main sites at risk from atmospheric NH_3 in the survey. In particular, sites with indicator values of more than double the threshold values included Bedlington, Glenmore Wood, Castle Ennigan, Orritor and Redgrave & Lopham Fen. Such results support the use of these measurements in an integrated programme of biomonitoring at sites.

- By contrast, sites with high wet deposition (Borrowdale, Yarner Wood) were not clearly identified using foliar total Nitrogen content and soluble NH_4-N concentrations. Although there is existing evidence that wet deposition does affect these parameters (e.g. Result from Whim Moss intensive study), the response appears to be smaller. Although it is not possible to determine from the chemical N signal alone, this differential sensitivity to NH_3 and soluble NH_4-N provides important information to help interpret results at sites where several N indicator methods are applied.

- More detailed examination of the foliar % N and soluble ammonium values at individual sites revealed a number of unexpected values. For example, at Knockan (one of the cleanest UK sites) values for *T. tamariscinum* were much higher than expected. This indicates that caution may be needed in such an extensive approach, which utilizes a simple collection of sample at one time. There is a case for more detailed checking of such values by more intensive sampling at particular sites.

11. UK Extensive Study: lichen diversity

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11.1. Introduction

This chapter reports the results of the extensive lichen diversity study, using macrolichens only, across the UK. The sites were sampled by conservation agency and SEPA staff. The sampling protocols and the UK site information are detailed in Section 9 (methods). Bark samples of trunk and twig were collected for pH determination at the Natural History Museum. Total and soluble NH_4-N concentration methods were tested in parallel with the moss sampling for total N deposition.

11.2. Results

11.2.1. Lichen diversity of macrolichens on trunks and twigs

A total of 43 macrolichen species were recorded, 35 of which were recorded on trunks and 33 on twigs (Table 11.1). Of these 15 were classified as acidophytes and 7 as nitrophytes according to van Herk (1999, 2002). Species occurring in this survey classed as acidophytes or nitrophytes are shown in Table 11.1. Species occurring over 100 times in all sites on trunks included 2 acidophytes *Cladonia coniocraea, Hypogymnia* spp. and *Parmelia sulcata, Parmotrema chinense* and one *nitrophyte Physcia tenella.* Species occurring over a 100 times in all sites on twigs included *Parmelia sulcata* and two nitrophytes *Physcia tenella* and *Xanthoria parietina.*

Table 11.1. Species recorded in the extensive UK survey

Species	Trunks	Twigs	A or N
Cladonia coniocraea	X		A
Cladonia chlorophaea	X		A
Cladonia macilenta	X		A
Cladonia polydactyla	X		A
Diploicia canescens	X		
Evernia prunastri	X	X	A
Flavoparmelia caperata	X	X	
Hypogymnia sp			A
Hypogymnia physodes	X	X	A
Hypogymnia tubulosa	X	X	A
Hypotrachyna laevigata	X	X	
Hypotrachyna revoluta	X	X	
Melanelia exasperata		X	
Melanelia exasperatula	X		
Melanelia glabratula	X	X	
Melanelia laciniatula		X	
Melanelia subaurifera	X	X	
Mycoblastus sterilis	X		
Parmelia saxatilis	X		A
Parmelia sulcata	X	X	
Parmelina pastilifera		X	
Parmotrema chinese	X	X	
Physia adscendens	X	X	N
Physcia aipolia		X	N
Physcia leptalea	X	X	N
Physia tenella	X	X	N
Platismatia glauca	X	X	A
Punctelia borreri		X	
Punctelia subrudecta	X	X	
Pseudevernia furfuracea		X	A
Ramalina calicaris		X	
Ramalina farinacea	X	X	
Ramalina fastigiata	X	X	
Rinodina sophodes		X	
Scoliciosporum	X		
Sphaerophorus globosus	X		
Usnea ceratina		X	A
Usnea cornuta	X	X	A
Usnea florida	X	X	A
Usnea subfloridana	X	X	A
Xanthoria candelaria	X	X	N
Xanthoria parientina	X	X	N
Xanthoria polycarpa	X	X	N

11.2.2. Tree species and bark pH

In the extensive sites it was not always possible to find acid barked trees so that lichens were recorded on other species where available. Tree species were used in the extensive survey including *Quercus* species (14 sites), *Betula* species (5 sites), *Fraxinus* (2 sites) *Crataegus monogyna* (2 sites), *Sorbus aucuparia* (2 sites), *Acer pseudoplatanus* (1 site), *Larix europaea* (1 site), *Populus nigra* (1 site) and *Picea sitchensis* (1 site). The results of lichens recorded on trunks are summarised in Table 11.2. Lichens on twigs of *Picea* (site 2) and on *Quercus* (site 3) were not sampled, the rest of the lichens identified on twigs are in Table 11.3.

Bark pH of both trunk and twigs were measured in the lab using a flat tip electrode (see methods 7.2). The results are shown in Table 13.2 together with acidophyte and nitrophyte data.

Table 11.2. Data for trunks across all sites including; mean bark pH, and lichen values for acidophyte mean (AV M) and standard deviation (AV StD), nitrophyte mean (NV M) and standard deviation (NV StD), Ellenber N mean (Ell-N M) and standard deviation (Ell_N StD) and diversity mean (Div M) and standard deviation (StD) The total deposition listed for each site in this table have been calculated for woodland irrespective of their habitat type.

					Total Woodland deposition					Trunk									
Site	Site name	Map ref.	NH_3 conc.	APIS SO_2	N dep.	NH_3-N dep.	S dep.	Rainfall	Tree										
no.			$\mu g\ m^{-3}$	$\mu g\ m^{-3}$	kg N ha^{-1} y^{-1}	kg N ha^{-1} y^{-1}	kg S ha^{-1} y^{-1}	Aver. Rain	spp.	pH	AVM	AVStD	NVM	NVStD	Ell M	Ell StD	AV-NV	DivM	Div StD
1	Cwmystwyth	SN 772743	2.96	1.5	50.1	32.2	15.52	1540.1	Qu	4.7	5.8	2.8	0	0	5.4	1.14	5.8	3.6	1.14
2	Plynlimon	SN822841	0.6	1.5	24.1	6.5	18.4	1939.0	Ss		14.4	2.3	0	0	4	1.41	14.4	2.8	0.84
3	Dyffryn Mymbyr	SH695572	1.18	1.3	47.5	13.1	23.36	3815.9	Ap	5.8	0.2	0.45	0	0	3.2	2.95	0.2	1.2	1.10
4	Stackpole	SR983950	1.81	2	28.4	18.3	12	1017.64	Qu	4.5	5.5	4.65	0	0	10	2	5.5	3.3	0.58
5	Eskdalemuir	NT235032	0.38	1.1	20.8	3.9	9.28	1555.7	Pn	5.4	0.83	1.75	0	0	10.6	4.83	0.83	2.8	1.10
6	Halladale	NC902488	0.83	1.2	17.3	8.6	12.48	1181.3	Bp	5.2	6.3	9.30	0	0	6	5.2	6.3	2	1.73
7	Strathvaich Dam	NH347750	0.18	0.6	13.4	1.9	20.16	1881.6	Bp	4.4	19	0	0	0	15	3	19	5	1.00
8	Glensaugh	NO664799	0.35	1.1	19.0	3.6	9.12	972.2	Sa	5.6	0	0	0.7	0.58	5.3	2.83	-0.7	1.3	1.00
9	Knockan	NC187088	0.12	0.8	8.1	1.2	11.04	1464.2	Sa	5.5	8.7	6.60	0	0	9	1.73	8.7	2.7	0.58
10	Edinburgh Centre	NT254738	2.5	16.6	43.5	25.8	24.8	910.7	Fx	5.3	7.7	5.69	0	0	4.67	3.06	7.7	2	1.00
11	Ariundle	NM835642	0.04	1.4	11.3	0.4	22.24	2608.2	Qu	5.8	0.4	0.55	0.8	1.79	3.4	3.44	-0.4	1	1.00
12	Glen Nant	NN014278	0.06	1.6	17.7	0.6	23.84	2587.7											
13	Wood of Cree	NX385715	0.12	1.5	16.2	1.2	12.8	1571.3	Qu	4.7	21.7	7.51	4.7	5.51	18.7	2.52	17	6	1.00
14	Bush	NT245635	2.19	3.7	40.3	22.6	12.8	1080.4	Qu		10.7	8.96	0.0	0	10.3	3.21	10.7	3.7	1.15
15	Ladybower	SK163905	0.73	5	51.5	4.2	30.56	1613.9	Bp	4.7	1	1.73	0.0	0	0.67	1.15	1.00	0.3	0.58

					Total Woodland deposition															
Site	Site name	Map ref.	NH_3 conc.	APIS SO_2	N dep.	NH_3-N dep.	S dep.	Rainfall	Tree	Trunk										
no.			$\mu g\ m^{-3}$	$\mu g\ m^{-3}$	kg N ha^{-1} y^{-1}	kg N ha^{-1} y^{-1}	kg S ha^{-1} y^{-1}	Aver. Rain	spp.	pH	AVM	AVStD	NVM	NVStD	Ell M	Ell StD	AV-NV	DivM	Div StD
16	Moorhouse	NY751334	0.44	2.2	39.6	4.8	20.64	1437.3	Le	3.9	19.5	1.00	0.0	0	3.5	1.73	19.5	1.5	0.58
17	Fenn's Moss	SJ478388	1.87	2.2	38.82	18.06	8.16	825.3	Qu	5.4	0	0	0.0	0	0.75	1.5	0	0.30	0.50
18	Stanford	TL858948	1.54	2.8	38.75	14.67	10.08	710.8	Qu	4.5	0.2	0.45	0.0	0	3.4	4.98	0.2	1	1.41
19	Fressingfield	TM261759	5.29	3.7	74.32	50.94	10.72	669.1	Qu	5.4	0.7	0.58	20.7	2.31	31	0	-20.00	6	0
20	Bedlington	TM173684	7.43	3.8	96.55	72.19	11.04	684.0	Qu	5.7	2.7	2.08	14.3	5.13	34	0	-11.6	6	0
21	Borrowdale	NY240135	0.19	2	40.10	2.02	28.64	2689.6	Qu	4.3	8	5.42	0.0	0	4.75	0.96	8.00	2	0.82
22	Brown Moss	SJ563390	3.95	2.7	57.91	38.31	8.96	803.4	Qu	5.4	0	0	5.0	1.58	9	4.24	-5.00	1.6	0.89
23	Llynclys	SJ273237	1.78	1.8	34.50	17.00	8	930.4	Bp	4.7	0	0	0.2	0.45	1.6	3.58	-0.2	0.4	0.89
24	Wytham Wood	SP452083	1.13	3.6	33.09	10.69	9.12	715.2	Qu	4	0	0	0	0	1.2	1.64	0	0.4	0.55
25	Lullington Heath	TQ538016	0.9	3.3	27.84	8.66	11.04	876.9	Cm	4.2	0	0	0	0	7.75	1.5	0	1	0
26	Lough Navar	H074545	0.47	0.6	11.48	4.48	9.76	1364.1	Bp	4.5	17.6	8.05	0	0	7.4	2.61	17.6	3	1.58
27	Glenmore Wood	H654608	2.28	0.7	32.49	21.99	8.8	969.6	Qu	4.5	0	0	0	0	0.8	1.79	0	0.2	0.45
28	Caldanagh Bog	D022205	2.58	1.5	36.33	25.13	9.6	1055. 6	Fx	5.4	0	0	4.80	6.57	4.8	6.57	-4.8	0.8	1.10
29	Castle Enigan	J121322	4.09	2	53.53	39.53	10.08	1043.5	Cm	4.9	0	0	0	0	0	0	0	0	0
0	Orritor	H768782	6.45	3.9	72.98	61.22	9.92	1183.5	Fx	5.7	0	0	0	0	0	0	0	0	0
31	Redgrave & Lopham Fen	TM050797	2.28	3.7	45.30	21.92	10.4	676.7											
32	Yarner Wood	SX786789	0.65	1.1	31.22	6.72	18.24	1634.8	Qu	4.3	11.50	2.38	0	0	8.60	3.87	11.50	3.2	1.17

Table 11.3. Data for twigs across all sites including; mean bark pH, and lichen values for acidophyte mean (AV M) and standard deviation (AV StD), nitrophyte mean (NV M) and standard deviation (NV StD), Ellenber N mean (Ell-N M) and standard deviation (Ell_N StD) and diversity mean (Div M) and standard deviation (StD).

Site	Site name	Map ref.	TWIG									
no.			Bark pH	AV M	AV StD	NV M	NV StD	Ell-N M	Ell-N StD	AV-NV	Div M	Div StD
1	Cwmystwyth	SN 772743	4.8	2.00	1.07	0	0.00	8.00	3.37	2.00	6.4	2.37
2	Plynlimon	SN822841										
3	Dyffryn Mymbyr	SH695572										
4	Stackpole	SR983950	5.1	5.5	4.65	2	0.82	19.5	8.96	3.5	9.2	2.87
5	Eskdalemuir	NT235032	5.2	1	0.7	4.6	2.07	32.4	5.9	-3.6	8.8	1.3
6	Halladale	NC902488	5.6	0.5	0.84	0.67	0.52	5.67	2.58	-0.17	1.6	0.81
7	Strathvaich Dam	NH347750	4.8	6.17	2.93	1	0.63	17.33	3.56	5.17	5.6	0.93
8	Glensaugh	NO664799	5.3	0.2	0.45	0.8	0.84	4.8	3.19	-0.6	2	0.7
9	Knockan	NC187088	5.3	0	0	1	0.82	7.43	2.15	-1	1.7	0.48
10	Edinburgh Centre	NT254738	6.2	0	0	0.33	0.52	3	3.95	-0.33	0.66	1.03
11	Ariundle	NM835642	5.2	5.6	5.94	0.8	0.84	14.2	7.16	4.8	8	1.6
12	Glen Nant	NN014278										
13	Wood of Cree	NX385715	6.5	0.22	0.67	0	0	0	0	0.22	5.1	0.78
14	Bush	NT245635	6.07	0.83	0.75	1	0.63	6.33	2.07	-0.17	1.33	0.81
15	Ladybower	SK163905	5.5	0	0	0	0	0	0	0	0	0
16	Moorhouse	NY751334	5	4.63	2.56	0	0	3.13	1.89	4.63	0	0
17	Fenn's Moss	SJ478368	5.9	0.6	0.55	10.2	3.56	27.6	2.88	-9.6	6	1.22
18	Stanford	TL858948	5.6	0.9	0.88	2.8	2.44	19.7	10.26	-1.9	4.4	2.27
19	Fressingfield	TM261759	6.1	0	0	10.4	4.83	24	2.83	-10.4	4	0.7
20	Bedlington	TM173684	5.3	0	0	6	2	10.8	2.68	-6	2	0
21	Borrowdale	NY240135	5.9	0.22	0.44	0.11	0.33	3.67	2.92	0.11	0.22	0.48
22	Brown Moss	SJ563390	5.8	0	0	9.9	3.28	13.2	3.79	-9.9	2.3	0.67
23	Llynclys	SJ273237	5.6	0	0	1.1	1.45	4.8	5.51	-1.1	1.6	1.17
24	Wytham Wood	SP452083	5.4	0.75	0.89	1.63	1.41	12	9.58	-0.88	2.2	2.06
25	Lullington Heath	TQ538016	5.2	15.5	4.14	1.67	1.51	17.67	6.06	13.83	6.3	1.63
26	Lough Navar	H074545	5.3	0.14	0.38	0.57	1.51	2.57	4.08	-0.43	6.6	1.71
27	Glenmore Wood	H654608	6	0	0	3	3.95	7.67	8.64	-3	1.8	2.04
28	Caldanagh Bog	D022205	6.1	0	0	9.5	4.45	10.8	3.79	-9.5	1.8	0.63
29	Castle Enigan	J121322	6.4	0	0	0	0	0	0	0	5.1	2.84
30	Orritor	H768782	6.4	0	0	0	0	0	0	0	1.8	1.48

Site	Site name	Map ref.	TWIG									
no.			Bark pH	AV M	AV StD	NV M	NV StD	Ell-N M	Ell-N StD	AV-NV	Div M	Div StD
31	Redgrave and Lopham Fen	TM050797										
32	Yarner Wood	SX786789	4.8	11.80	6.92	1.33	1.03	30.67	8.19	10.47	10.5	2.42

11.2.3. Nitrophyte and acidophyte values on trunks and twigs

Six sites without either acidophyte or nitrophyte values were excluded from the data set for this assessment. These included 3 sites on *Quercus* (17, 24 & 27), 2 on *Crataegus* (25, 29) and one on *Fraxinus* (30).

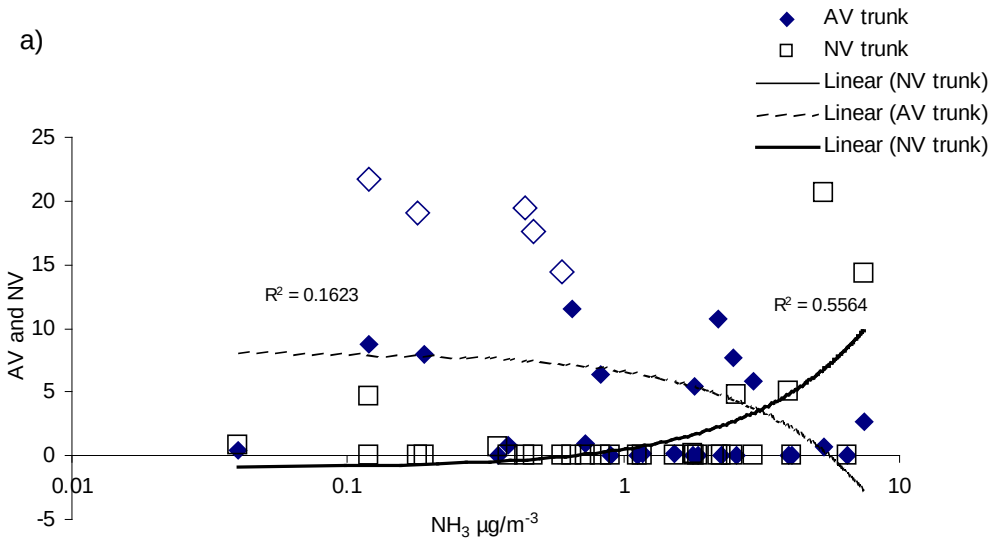

Figure 11.1. Acidophyte (AV) and nitrophyte (NV) values on a) trunks and b) twigs for all sites plotted against log NH_3 concentration µg m^{-3}. In a) open diamonds on trunks are upland sites 2, 7, 13, 16 and 26. Highest nitrophyte values were above 14 at sites 19 and 20. In b) values for AV above 10 were on *Quercus* at site 32 (Yarner wood) and on *Crataegus* at site 25 (Lullington heath).

For all sites sampled there is a better correlation of nitrophyte values than acidophyte values with log ammonia concentration (Figure 11.1). However sites with solid diamonds are high altitude and high rainfall sites on acid barked species and in these sites nitrophytes are absent except on oak at site 13. Conversely the negative AV values on twigs in many sites contribute to the low R^2. At Lullington Heath there were no acidophytes on *Crataegus* trunks but a very high AV of 15.5 on twigs, as twigs of this species are often colonised very rapidly.

Bark pH is known to affect lichen communities making it difficult to compare sites with trees of differing species and bark pHs. If acidophyte and nitrophyte values for all samples on oak are plotted against ammonia concentrations acidophyte values still show little correlation with NH_3 concentration especially at higher concentrations where acidophyte values are zero (Figure 11.2).

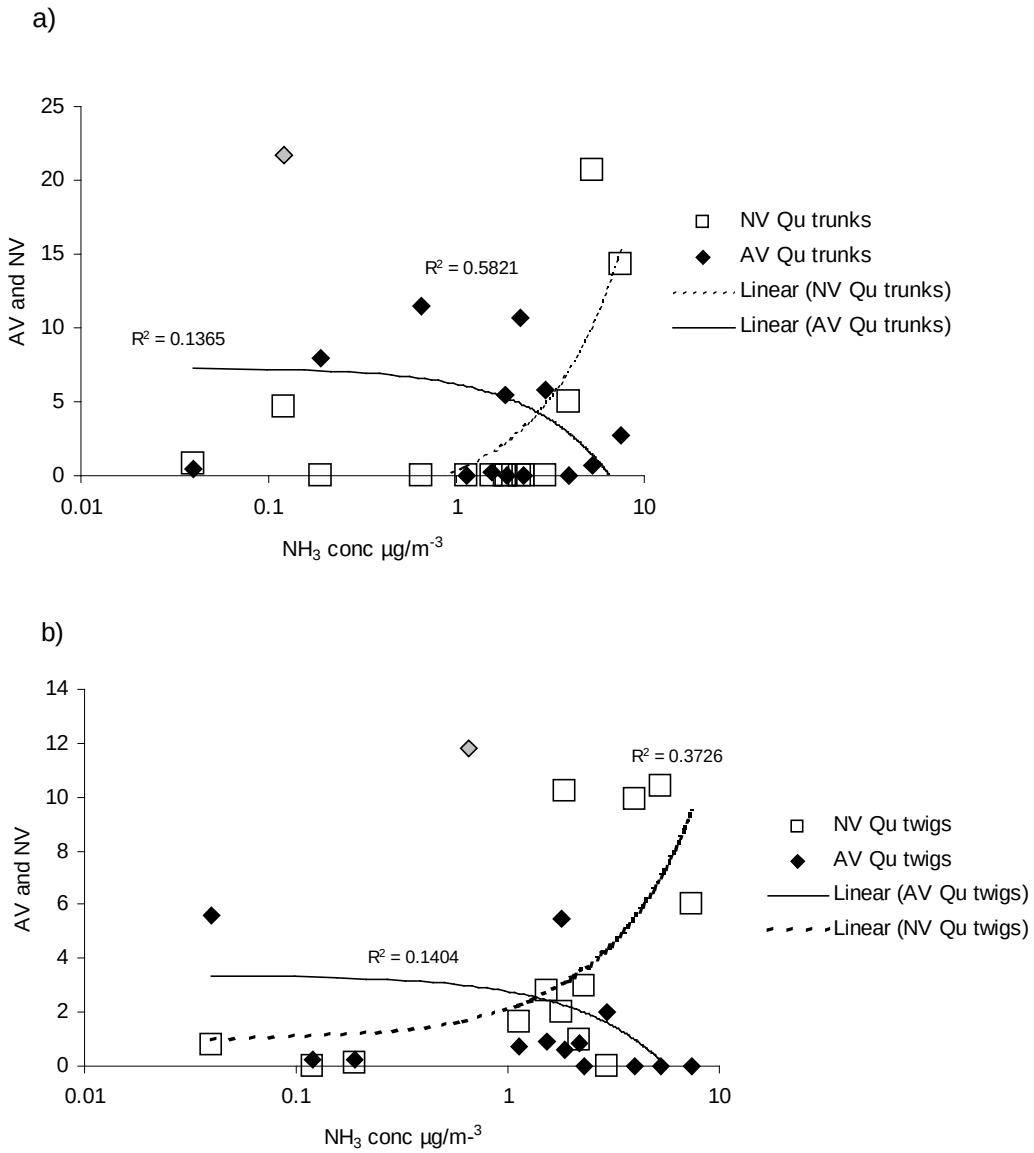

Figure 11.2. Acidophyte (AV) and nitrophyte (NV) values for oaks (Qu) at all sites sampled a) on trunks and b) on twigs showing better correlation of nitrophytes with ammonia concentrations than acidophytes. Loss of acidophytes on twigs is occurring at lower concentrations of ammonia. On trunks the solid diamond is site 11 Ariundle and on twigs site 32, Yarner Wood.

The British Isles have a strong climatic gradient from east to west from continental conditions with low rainfall to oceanic conditions with high rainfall, causing leaching. This is associated with lower bark pH and an increasing number of natural acidophytes. If acidophyte values for If acidophyte and nitrophyte values for trunks and twigs are plotted against NH_3 concentrations the linear correlations are very similar for both AV and NV values, but the loss of acidophytes is occurring at lower concentrations on twigs than on trunks, with the exception of site 11 Ariundle on *Quercus* with unusually high acidophyte values (Figure 11.2). If acidophyte values for all sites are plotted against rainfall the increase in acidophyte values with rainfall occurs up to 1700 mm pa, however above 2000 mm pa acidophyte values are low. At both lower and higher rainfall levels other factors must influence the distribution of acidophytes, e.g. bryophyte dominated communities at higher rainfall (Figure 11.3).

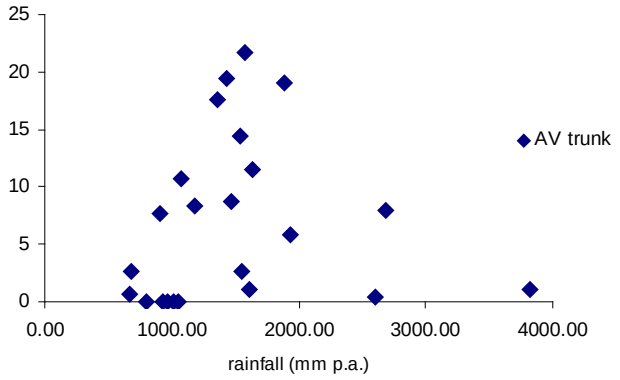

Figure 11.3. Acidophyte values plotted against rainfall at all sites showing that at low rainfall and very high rainfall other factors are influencing the acidophyte lichen values.

The effect of bark pH on acidophyte and nitrophyte distribution is shown in Figure 11.4. At bark pHs between 3.5 and 4.5 acidophyte values are high and nitrophyte values are either 0 or very low, whereas at pH's >5 acidophyte values drop rapidly whilst nitrophyte values increase rapidly.

Bark pH varies considerably with each tree species, and without a good data set from each tree species in a specified location (as in a transect from a source), it is not possible to detect the increase in bark pH associated with ammonia (Figure 11.5). Oak was the preferred species and 13 sites were sampled for oak. These show a tendency towards increased bark pH at levels above 1µg/m^3 but other factors may affect pH of the bark such as tree age, rainfall in oceanic sites (e.g. Ariundle) or geology in calcareous sites. This makes it difficult to use bark pH as a method across a range of sites.

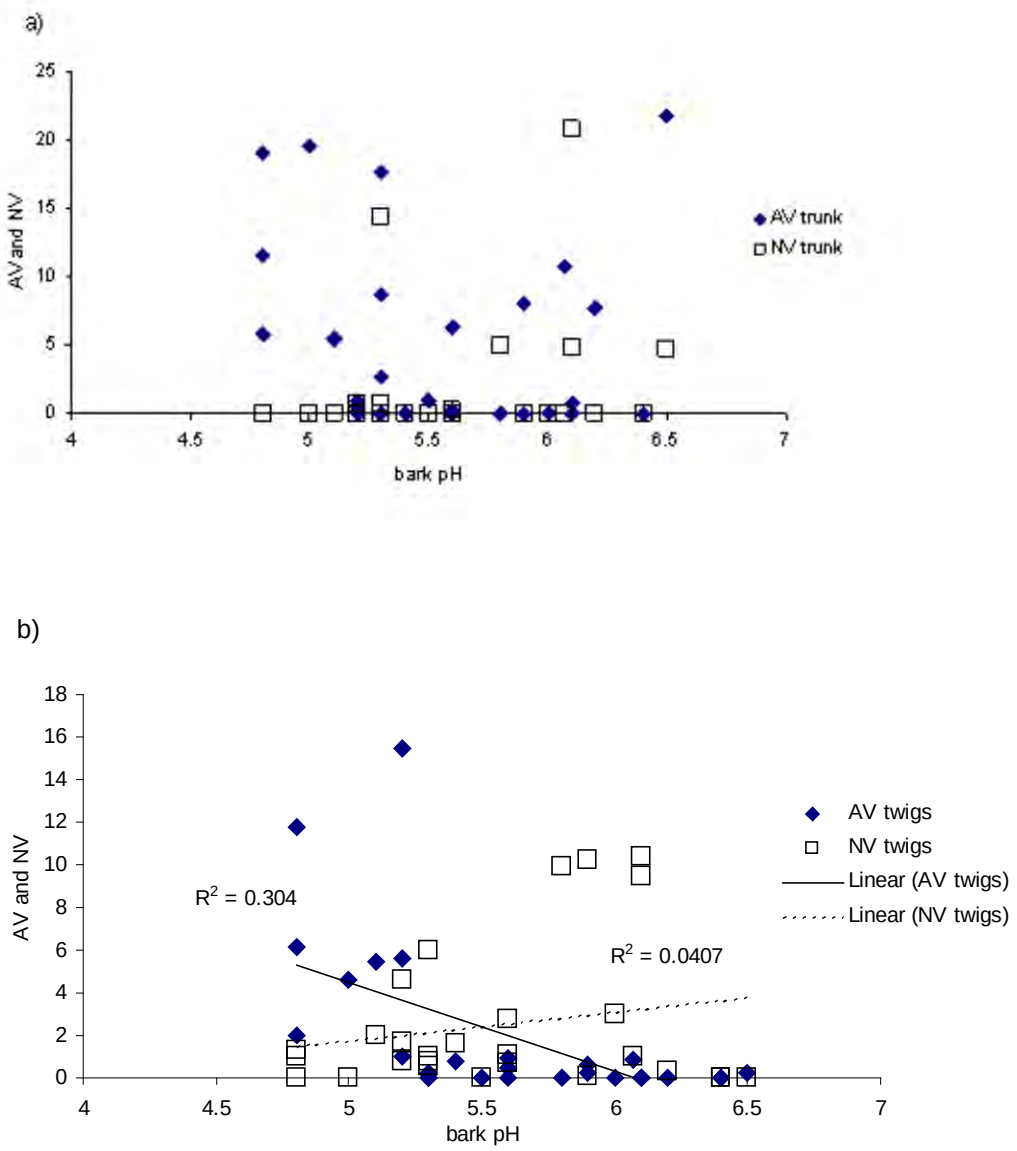

Figure 11.4. Acidophyte (AV) and nitrophyte (NV) values on all trees for a) trunks and b) twigs plotted against bark pH showing rapid change between acidophytes and nitrophyte values between pH 5 and 5.7 for both trunks and twigs.

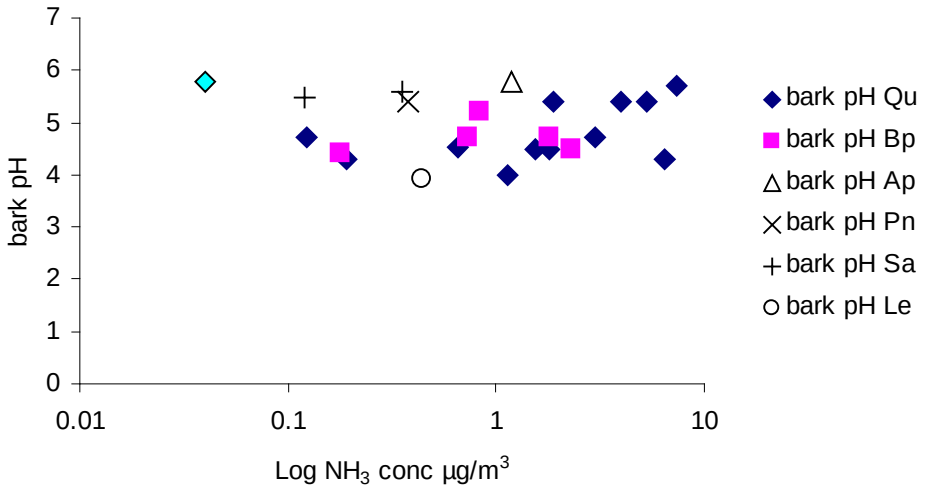

Figure 11.5. Bark pH shows little correlation with ammonia concentrations at all sites across the UK. The considerable variation in tree species contributes to this. Site 11 on *Quercus* at Ariundle is distinguished by a light coloured diamond.

11.2.3. Combining the results of lichens with bark pH and N deposition

It should be noted that the data also suggest an interactive response to both bark pH and nitrogen supply. This needs to be further investigated, but initial analysis using multiple regression shows that the correlations may be increased considerably by considering the dual effect of bark pH and N supply on the lichen signal, as measured by the combined score of AV-NV. This is illustrated in Figures 11.6 and 11.7, for lichens on trunks and twigs respectively. In both cases a much higher R^2 is found with NH_3 concentration than with N deposition.

The data show slightly higher correlations between AV-NV and a combination of N deposition / S deposition ratio and bark pH (not shown). However, caution is needed in attributing the cause of the effect to either NH_3 or N deposition/S deposition ratio. This is because the N/S ratio is highly correlated with NH_3 concentration at the sites across the country ($R^2 = 0.85$), much higher than the correlation with any other pollutant parameter. Thus it cannot be said strictly if the NH_3 or the N/S ratio is the functional driver. However, since the NH_3 concentration remains the main driver of the N/S ratio, in practice it is NH_3 levels that control the signal.

It is interesting that the same multiple regression can be used to combine the twig and trunk lichen datasets, which is shown in Figure 11.8 for lichens on oak. The two datasets are fully superimposed, implying that the difference between the trunk and twig lichen values can be well explained simply on the basis of their different bark pH values. The combination of the two methods is therefore very useful in improving robustness since it a) provides two independent estimates and b) tends to use different lichen communities to derive because of the different bark pH values.

It is interesting in the UK dataset that there is no significant correlation between trunk pH and NH_3 or N deposition, although there is a weak correlation ($p=0.04$) between twig pH and NH_3 concentration. This suggests that the effect of N on epiphytic lichens is not just mediated by an effect of NH_3 on bark pH (as suggested by the transect studies and a landscape assessment in East Anglia, Sutton *et al*. 2004a; Wolseley and James 2002b). Rather, the UK data appear to show an independent direct effect of N in addition to that mediated through changes in bark pH. Van Herk (2001) also noted a similar response in the Netherlands.

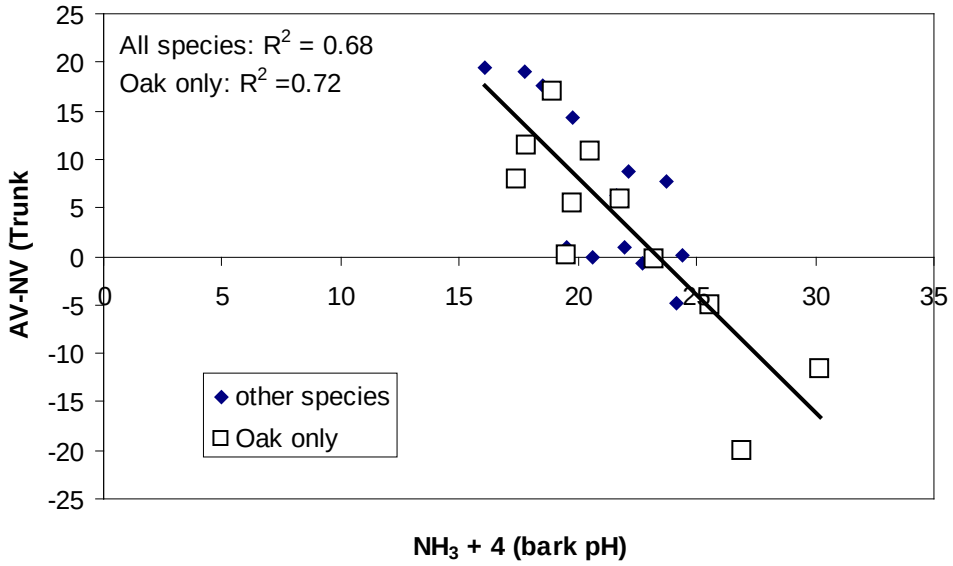

Figure 11.6. Multiple regression between overall trunk epiphytic acidophyte and nitrophyte lichens (AV-NV) and the combination of measured ammonia concentration (μg m^{-3}) and measured trunk pH. The regression is plotted for the full dataset.

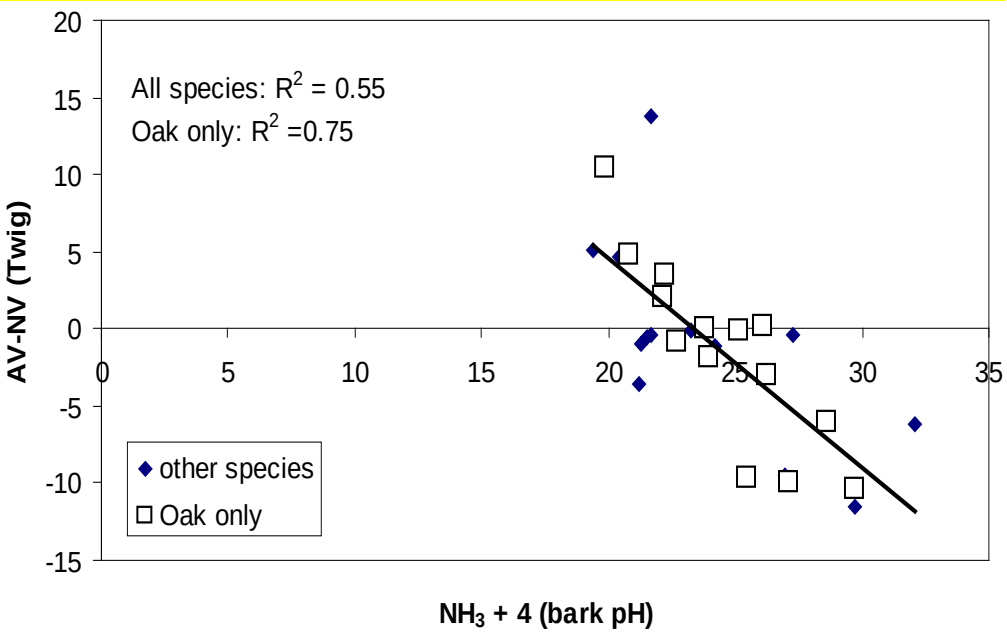

Figure 11.7. Multiple regression between overall trunk epiphytic acidophyte and nitrophyte lichens (AV-NV) and the combination of measured ammonia concentration (μg m^{-3}) and measured trunk pH. The regression is plotted for the full dataset.

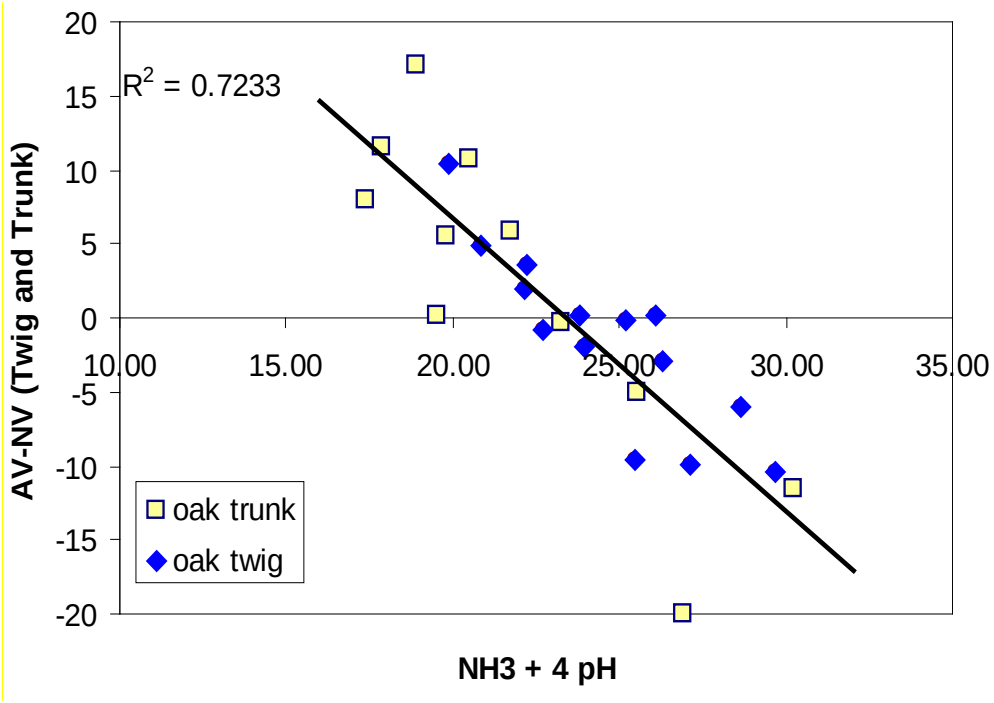

Figure 11.8. Multiple regression between epiphytic acidophyte and nitrophyte lichens (AV-NV) on oak for trunks and twigs and the combination of measured ammonia concentration (μg m^{-3}) and measured trunk or twig pH, respectively.

11.2.4. Ellenberg values

Ellenberg N values for trunks also show a linear correlation with NH_3 concentrations. Sites falling above the linear correlation include site 5 on *Populus nigra*, 7 on *Betula* spp., 13 and 22 on *Quercus* at Brown Moss (Figure 11.9). The latter site is in a region of former acidification.

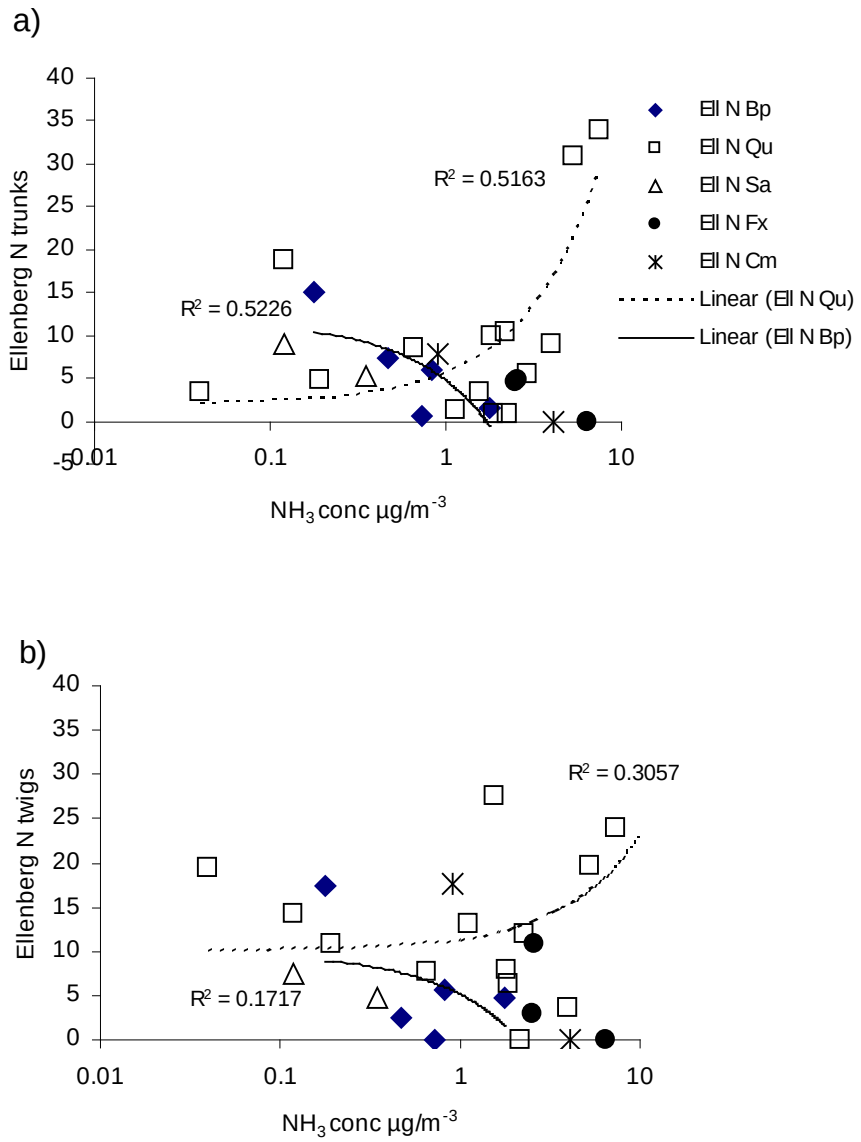

Figure 11.9. a) Ellenberg N values on trunks for all sites showing a linear correlation with ammonia concentration of c. 0.52 for *Betula* (Bp) and *Quercus* (Qu) corresponding well with the nitrophyte value. b) Ellenberg N values on twigs of all species show a lower correlation on *Quercus* and *Betula*. Other species : *Sorbus aucuparia* (Sa), *Fraxinus* (Fx), *Crataegus monogyna* (Cm), *Populus nigra* (Pn).

11.3. Comparison with other pollutants

The study has shown a good correlation with ammonia and nitrophytes across the UK but the correlation with acidophytes is not as good due to a) a strong climatic gradient and b) the species selected as indicators.

In order to test whether other pollutants were correlated with distribution of acidophytes and nitrophytes across UK, AV and NV for all samples were plotted against SO_2 concentration, S deposition and against total N deposition (Figure 11.10). The results demonstrate that nitrophytes are not tolerant of S deposition > than 15 kg ha^{-1} y^{-1} and that acidophytes are tolerant of S deposition between 10 and 20 kg ha^{-1} y^{-1}, with a relatively normal distribution

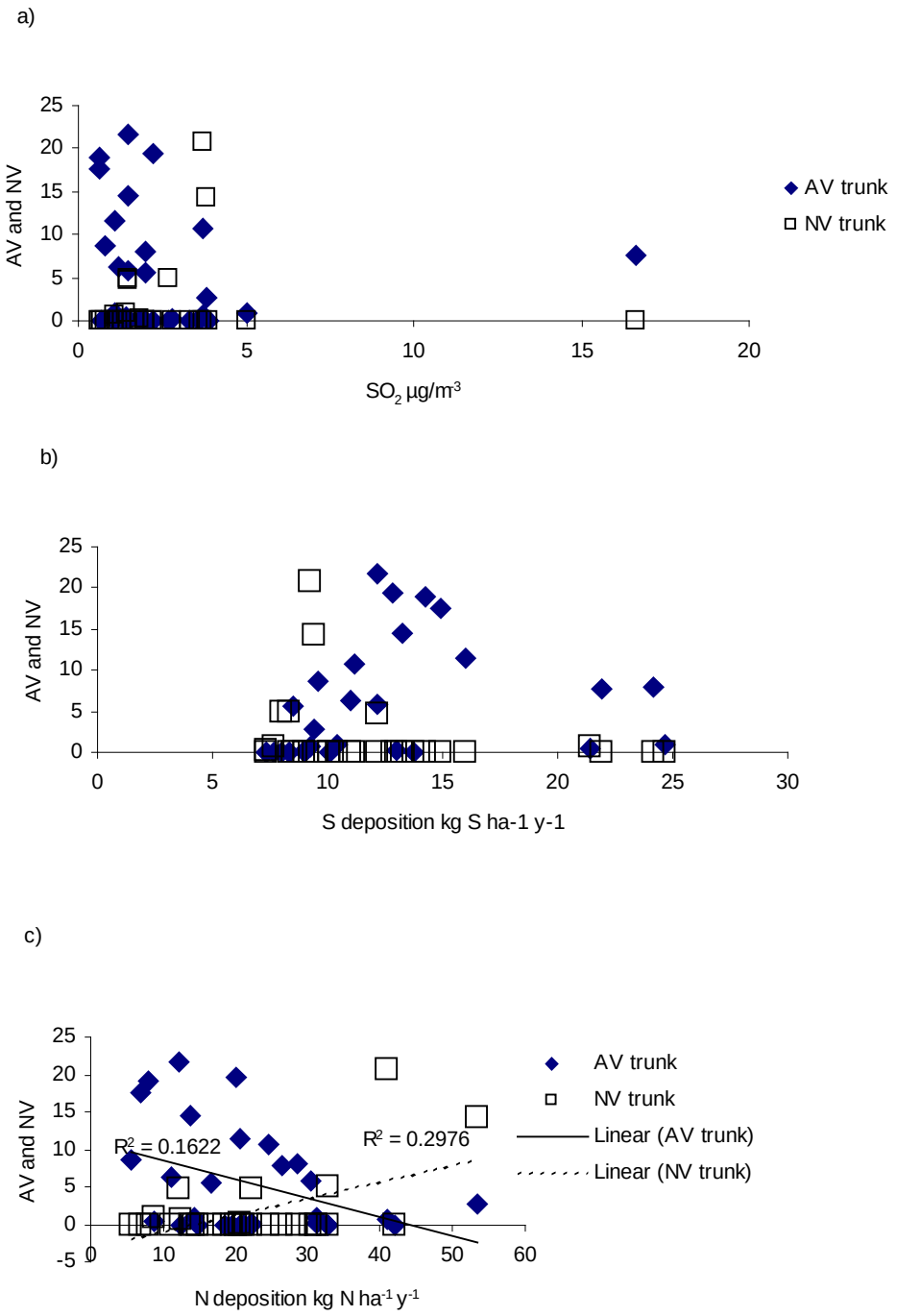

Figure 11.10. Mean acidophyte (AV) and nitrophyte (NV) values for trunks in all sites plotted against a) SO_2, b) S deposition and c) total N deposition kg ha^{-1} y^{-1}.

with highest AV values of 22 at c. 12 kg ha^{-1} y^{-1}. A similar pattern of acidophyte values was seen with total N deposition while the nitrophyte values were exponentially increasing at 100 kg ha^{-1} y^{-1}.

11.4. Comparison of biomonitoring methods applied across the UK

11.4.1. Site and tree species variation

The variation in habitat and available tree species contributed to the range of factors other than ammonia or total N deposited affecting lichen communities. Oak (*Quercus*) was the preferred tree species with a low bark pH as this species was used across the extensive survey in the Netherlands (van Herk 1999, 2002). Tree species with a high bark pH such as *Fraxinus excelsior*, *Populus nigra* and *Acer pseudoplatanus* are not suitable for evaluating ammonia concentrations as they frequently carry nitrophytes and in continental conditions of low rainfall rarely carry acidophytes. Although conifers were used on sites for the intensive survey and acidophyte values have been shown to respond to ammonia concentrations at Whim Moss and Happendon no nitrophytes are present on these trees on trunks or twigs. In addition, softwood plantations in upland areas are not appropriate especially in areas where epiphytic lichens are scarce or absent (e.g. upland moorland). Low values in these cases may be attributed to absence of propagules.

11.4.2. Bark pH

Bark pH was measured on trunks and twigs (Section 7) and has been widely used as an indicator of increasing ammonia concentrations in the Netherlands (van Herk 1999, 2002) and more recently in Britain (Wolseley and James 2002a; Sutton *et al.* 2004b). Where the same tree species is available throughout the transect or where climatic conditions are more or less homogeneous, increasing bark pH has been correlated with increasing atmospheric ammonia (van Herk 1999; Section7.). However in a UK survey there is almost no correlation of bark pH with ammonia concentrations of either trunks or twig samples due to the range of tree species and climatic conditions across the sampling sites.

11.4.3. Acidophyte, Nitrophyte and Ellenberg N values

Both van Herk (1999, 2002) and Wirth (1992) developed indicator lists of epiphytic lichens that included crusts and macrolichen species. In this study we have confined sampling to macrolichens only. This reduces the number of indicators applied so that indicator scales are lower than when all species are used.

In all sites where specimens were recorded and data available there was a reasonably good correlation of reduced nitrophyte and reduced Ellenberg-N values with ammonia concentrations. This is due to the widespread occurrence of species of *Physcia* and *Xanthoria*.

Acidophyte species are defined by van Herk for continental Europe and were shown to correlate negatively with increasing ammonia concentrations. However in Britain there is a strong gradient in acidophyte species from the continental conditions of the lowland east to the oceanic conditions of the west. This is associated with increased rainfall and with occult precipitation, particularly in upland areas. This gradient is obvious in our results where acidophyte values correlate with rainfall between 500–1700mm, but above and below that range other factors influence the distribution of lichen communities.

Acidophytes are abundant in the west of Britain and many of our rarer epiphytic species are either acidophiles or within acidophilous communities. It is therefore important to assess changes in acidophyte values that may be associated with loss of condition in sites of conservation importance. Their negative correlation with increasing ammonia concentrations demonstrates their susceptibility to ammonia and to long-range deposition of ammonium (van Herk 2003). The acidophyte community in Britain contains many additional species that do

not occur in continental Europe and this makes our adoption of the van Herk list of acidophytes inappropriate without further analysis of species distribution and climatic and pollutant data. This would enable greater precision in the use of acidophyte values for assessing changes in condition.

The combination of acidophyte and nitrophyte values can be used to assess changes over time within a site. At Tycanol NNR in west Wales sites were established on oak in pasture, moorland and woodland glade sites in 1999 and repeated in 2003. Nitrophytes had increased in pasture sites and *Xanthoria polycarpa* appeared for the first time, but the loss of acidophytes was marked in all sites. Analysis of total N in thalli of *Hypogymnia physodes* from all sites showed that total N was lowest in the protected glade site and highest in both pasture and exposed moorland sites (Larsen *et al.* 2005). In the absence of ammonia or other data on N deposition the results suggest that changes in condition of this site are associated with Nitrogen deposition in some form.

11.4.4. Trunks and twigs

The value of recording on both twigs and trunks has been demonstrated on this extensive survey. The succession of lichen communities with age of bark is well known (James *et al*.1977) so that a comparison of trunks of trees of different ages from different habitats may provide conflicting evidence. The development of the Indices of Ecological continuity in woodlands (Rose 1976, Coppins and Coppins 2002) is associated with the distribution of veteran trees and habitats where there is little environmental change over long periods of time. In the absence of lethal pollutants lichens on tree trunks may change slowly in altered conditions. In contrast, twigs present a new substratum each year, which is colonised by propagules from surrounding lichen communities. If the bark substrate is altered by deposition of nitrogen in a basic form acidophyte species are unable to grow while nitrophyte species appear (Wolseley and Pryor 1999). The value of sampling both communities allows a prediction of factors that are changing with time, as in Devon in the vicinity of a farm yard where acidophyte values were high on trunks, while nitrophyte values were high on twigs and acidophytes almost absent (Wolseley and James 2002a). This method would allow interpretation of changes within and on the margins of designated conservation sites as well as condition assessment for designated species.

11.5. Conclusions

- Macrolichens can be used as indicators of atmospheric ammonia concentrations where appropriate acid-barked tree species occur.

- Atmospheric ammonia concentration is correlated with both N values and Ellenberg N scales on trunks and twigs, but the use of selected species as nitrophytes gives a more precise relationship.

- Acidophyte values are correlated with increasing ammonia concentrations at lower R^2 due to the effect of climatic factors such as rainfall and altitude.

- Loss of acidophytes is occurring at lower ammonia concentrations than increase in nitrophytes and this effect is more conspicuous on twigs.

- In order to improve the use of acidophyte values as sensitive indicators of changes in atmospheric Nitrogen deposition across Britain climatic factors, especially rainfall and occult precipitation, need to be taken into account. This requires multivariate analysis of the extensive data set to establish species contribution in different conditions.

- The combination of acidophyte and nitrophyte values on trunks and twigs provides an index of N where positive values are associated with increasing acidophytes and negative values increasing nitrophytes and increasing ammonia concentrations. A comparison of the N index on trunks and twigs may allow detection of changes in response to changes in ammonia concentration over time. This method can be used for site condition assessment in appropriate sites.

12. Extensive UK scale study: Comparison of expert and non-expert sampling

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12.1. Introduction

Two sites were selected for a comparison of expert with non-expert sampling, Bush Estate in Midlothian (site 14) and Stackpole NNR in Pembrokeshire (site 4). Non-expert sampling included epiphytic macrolichens only and expert sampling included crustose and endophloedal epiphytic species. At Bush Estate, non-expert sampling was conducted by Ian Leith and Netty van Dijk. At Stackpole NNR, non-expert sampling was conducted by students on a lichen course at Orielton Field Centre. As they were all identifying crustose as well as machrolichens, we did not separate the data sets, but any doubtful specimens were resolved. In addition other data collected by expert lichenologist Ray Woods at Cwmystwyth (site 1), Ceredigion, West Wales and at Yarner Wood NNR (site 32) by Pat Wolseley is compared for total species and macrolichens only.

12.2. Methods

The same methods were used as those performed for the extensive site survey (described in Section 9) along with sampling forms set out in Appendix III. However, the expert sampling included all species present while the non-expert sampling only included macrophytes. Bark and twig specimens were collected and analysed for pH at the Natural History Museum (NHM). Bush Estate was surveyed by Pat Wolseley in May 2004 when trees were tagged for re-sampling by non experts, which was done in October 2004. Twigs were not marked so that twig sampling was not identical. This was the only site that was done at different times and independently of a lichenologist. At the Stackpole site course participants had already received training in identifying lichens on trunks and twigs. Pat Wolseley was tutor on the course and as the site was not easily accessible without a guide, both expert and non-expert sampling was done at the same time, with species identified *in situ* once appropriate trees had been selected. At Cwmystwyth and Yarner Wood sampling was done at the same time and the data treated for macrolichens only and for all lichens found.

12.3. Results

At all sites native *Quercus* species were used as the sampled tree for trunks and twigs. However conditions around the trunks varied considerably. At Bush Estate trees were in an open parkland, whereas at Stackpole the trees were in rather dense regeneration on calcareous rock, where it was difficult to find enough oak trees and only one had an un-shaded trunk. At Cwmystwyth, the trunks were shaded from a plantation and dominated by bryophytes. This was obvious on the form in the section where bryophyte cover was noted to occur on average in 16/20 quadrats. At Yarner Wood the trunks were well exposed along an old field bank at the woodland margin.

The results are compared for trunks and twigs for species diversity, and values for acidophyte, nitrophyte and Ellenberg-N (Table 12.1).

Table 12.1. A comparison of 4 sites sampled for total lichen diversity (tot) and macrolichen (mac) diversity at Bush by experts (Ex) and non experts (Ne) on trunks and twigs of *Quercus* for mean species diversity (div), acidophyte value (AV), nitrophyte value (NV) and Ellenberg N.

	S 14 Bush		**S 4 Stackpole**		**S 1 Cwmystwyth**		**S 32 Yarner Wood**	
	Ex (tot)	**Ne (mac)**	**tot**	**Ne (mac)**	**tot**	**mac**	**Tot**	**mac**
NH_3 conc ug m^{-3}	2.19	2.19	1.81	1.81	2.96	2.96	0.65	0.65
Rainfall (mm)	1080	1080	1018	1018	1540	1540	1635	1635
Div Trunk macros	4.7	3.7		3.3		3.6		3.2
Div Trunk tot	6.3		6		3.6		6.8	
Div Twig macros	3.7	2.2		9.25		6.43		10.5
Div Twig tot	5		12.75		6		12.67	
AV Trunk	10.7	10.7	1.3	1.3	5.8	5.8	13.9	11.5
NV Trunk	0	0	0	0	0	0	1.33	1.33
AV Twig	0.75	1	5.5	5.5	1.7	1.7	7.8	7.8
NV Twig	7	1.17	1	1	0	0	1	1
Ellen. N Trunk	15	10.3	19.7	10	14.8	4.9	17.2	8.6
Ellen. N Twig	16.25	6.33	28.25	19.5	10.86	8	34.3	30.67

12.3.1. Diversity on trunks and twigs

Bush Estate was the only site that was sampled at different times by expert and non experts thus enabling a full assessment of expert and non-expert results. At this site there was a difference in the macrolichen sampling averages for both trunks and twigs (Table 12.1), but a greater difference when crustose species were included in the total diversity. Total diversity was highest on the well-exposed trunks at Yarner Wood, then at Bush Estate and Stackpole, with a considerable drop to the densely shaded bryophyte-dominated trunks at Cwmystwyth. Twig diversity reflects the environmental conditions at each site with Bush Estate having lowest diversity of both macros only and all species, and Yarner Wood and Stackpole having similar diversities of macros and all species. Of these sites Yarner Wood has the lowest NH_3 concentration and total N deposition (Table 10.2) and all sites are below the critical level.

12.3.2. Acidophyte and nitrophyte values

Sampling of trunks and twigs for macrolichen and total acidophyte and nitrophyte species defined by van Herk (1999) for continental Europe has been applied to sites which are all very different from the lichen flora of continental Europe, three sites being in the far west with high rainfall and one site being in the southwest of Edinburgh. Acidophyte values were highest at Yarner Wood for both expert and non-expert results where NH_3 concentrations were lowest and also coincided with highest rainfall. Few of van Herk's crustose indicators are regarded as widespread in Britain whereas the macrolichen indicators include widespread species including whole genera such as *Cladonia* and *Usnea* as indicators. This provides a broad indicator basis, which shows better correlation with increasing numbers of species.

Nitrophytes were absent from all sites on trunks except Yarner Wood, whereas they occurred at low frequency on twigs in all sites except Cwmystwyth (Figure 12.2). The differences in values between expert and non-expert sampling at Bush Estate are due to differences in the estimates of frequencies of nitrophyte species on twigs. This difference is exaggerated if a species is found on all three sections of the twig by doubling the figure. The expert will search the girdle scars to find small thalli of nitrophytes, which were not easy to identify by the non-expert!

The expert sampling of all species at Stackpole NNR included a range of crustose and endophloedal species that are characteristic of young twigs in areas where there is no competition from algae. These species are important indicators of a healthy twig community that is not affected by Nitrogen. Stackpole is on a limestone substrate but there were no nitrophytes on the trunk and few on the twigs.

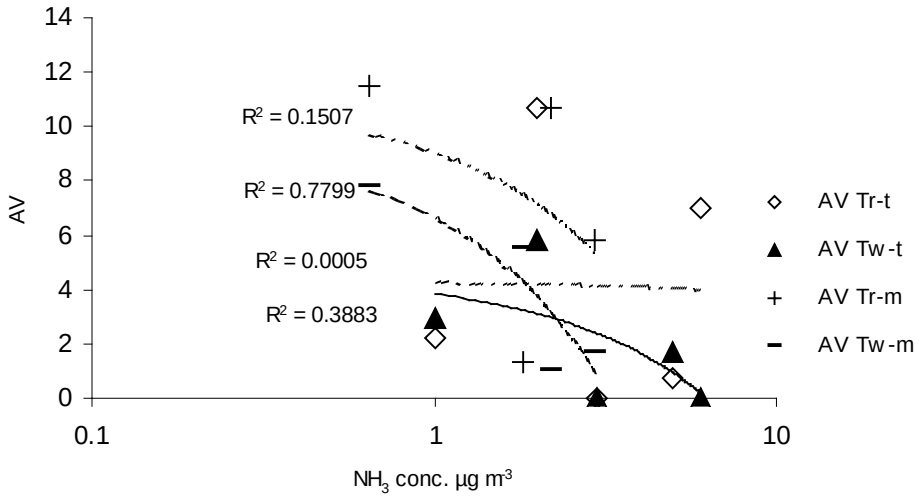

Figure 12.1. Acidophyte values for macrolichen (m) and total diversity (t) plotted against log NH_3 concentration µg m^{-3} showing that macrolichen acidophyte values on twigs are better correlated with NH_3 concentration (R^2=0.7799) than total acidophyte species (R^2= 0.3883) and that on trunks the correlation of acidophytes with NH_3 was low (R^2= 0.1507) using all species and lower (R^2= 0.0005) using macrolichen species.

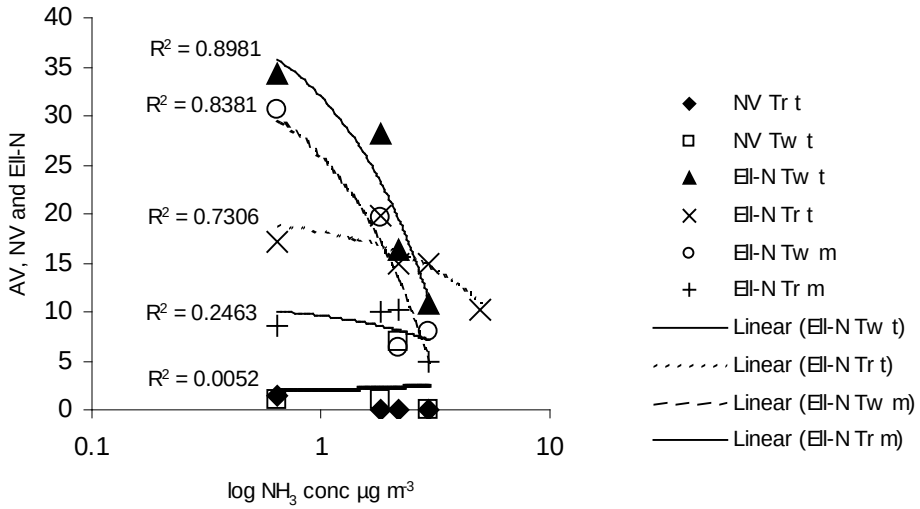

Figure 12.2. Nitrophyte values (NV) and Ellenberg -N values (Ell-N) from macrolichen (m) and total species (t) surveys plotted against log NH_3 µg^- m^{-3} showing better correlations for trunks than twigs using Ellenberg N with all values falling with increasing NH_3 and very low nitrophyte correlations on twigs due to absence of nitrophyte species.

At Yarner Wood there are nitrophytes on both twigs and trunks. Former research has shown that acidophytes may persist longer in areas of high rainfall (exceeding 1500 mm per year.) where there is considerable leaching of the bark (Wolseley and James 2002b). In contrast the high values of acidophytes and the absence of nitrophytes on tree trunks at Bush may in part be due to a history of former acidification in this area. The frequency of nitrophytes on the twigs at Bush in the expert valuation suggests that this is no longer the case.

Acidophyte values on trunks show very low correlations with NH_3 for both total and macrolichen sampling, but on twigs there is a marked difference in correlation of the macrolichens with NH_3 concentration and that of total species due to there being a greater number of macrolichen acidophytes present on the twigs. At Bush Estate the difference in AV values for expert and non-expert sampling was largely due to differences in estimates of frequency (Figure 12.1). Small specimens are not always easy to see on the youngest portion of the twigs; however in the scoring if the species occurs on all 3 intervals of the twig then the score is doubled.

Nitrophyte values are low in all four sites on both twigs and trunks and the low values coincide with few indicator species and differences in frequency estimates between expert and non-expert sampling on the twigs, with the drop in nitrophyte values where NH_3 concentrations are lowest and rainfall highest at Yarner Wood in the SW (Figure 12.2).

12.3.3. Ellenberg N values

The Ellenberg N values show a strong negative correlation with increasing ammonia for both macro lichen and total species (Figure 12.2). Ellenberg values between 1-9 are assigned to all species so that component species contribute to the total value while van Herk acidophytes and nitrophytes are selected as exclusive indicators. If only macrolichen species are included in the sampling, the Ellenberg N values are much lower than when based on total sampled epiphytic diversity. This means that sites where only macrolichens are recorded will have lower values than those where all species are recorded and that sites with higher diversity will tend to have higher values than those with low diversity.

In the UK survey Ellenberg N values have provided better correlations with NH_3 than van Herk indicators. However the Ellenberg scores are obtained from a large number of species while indicator species were selected from results in the Netherlands showing a good correlation with NH_3 concentration. This was most conspicuous for species on twigs, which were excluded from the Netherlands data. The inclusion of indicator values for a large number of species contrasts with the selected acidophyte and nitrophyte indicators of continental Europe. However, values assigned to lichen species for N deposition are also not tested in the UK climatic conditions and Wirth (1992) suggests that Ellenberg values should be tested before use outside the region for which they were devised.

12.4. Discussion

12.4.1. Expert and non-expert use of indicator values

Using the four test sites where the results of the macrolichens only survey were compared with the results of expert recording of all lichen species, macrolichens were found to show a good correlation with NH_3 concentration on twigs using van Herk's acidophyte and nitrophyte indicators, but the correlation on trunks is very low. This is partly due to the exclusion of crustose indicator species that are included in the van Herk list and to the frequency of widely distributed macrolichens, which are also readily identifiable. The Ellenberg N values show a strong negative correlation with increasing NH_3 concentration on twigs and a lesser one on trunks. The dissimilarity between total and marcrolichen Ellenberg N values is due to a larger number of species contributing. The Ellenberg values (Wirth 1992) are also devised for continental Europe and the species list excludes both recently described species and many species that are more frequent in western climates of Britain including widely distributed species such as *Fuscidea lightfootii, Lecanora confusa,* sensitive species of *Usnea (U. ceratina, U.cornuta*) and old forest species *Dimerella lutea*.

12.4.2. Selection of indicator species

The use of indicator species selected for a continental climate is of limited value when applied across a range of climatic conditions in the UK. The stronger negative correlation of acidophytes on twigs with NH_3 concentrations is emphasised in the western sites of Yarner Wood, Stackpole and Cwmystwyth, where continental nitrophytes are infrequent. However, the results of the macrolichen survey suggest that there is a good correlation of both acidophyte and nitrophyte lichen communities with increasing nitrogen, but that indicator species need to be assessed against climatic and pollution conditions in the UK in order to provide appropriate indicator lists for use in the UK.

Recent work in local areas has shown that there is a good correlation between a decrease in acidophyte values with increasing NH_3 concentrations (Sutton *et al*. 2004, Wolseley & James 2004) but the present UK survey has shown that this will vary across the UK due to a climatic gradient where increasing rainfall or occult precipitation may have an ameliorating affect on forms of N precipitation (see Section 11). Recent research has also indicated that the loss of acidophytes may be taking place at lower concentrations than currently established critical levels (Sutton *et al.* 2004a), and as many of our sites of conservation importance are associated with species-rich acidophyte communities it is most important to establish relevant and reliable indicator values for Britain. Indicators for acidophyte and nitrophyte communities in the UK can be assessed using multivariate analysis of a data set that includes macrolichen species and environmental factors of the UK survey sites.

12.4.3. Twig v. trunk

Lichen communities on twigs show a better correlation with NH_3 concentrations than those on trunks and also show less difference between macrolichen and total species sampling. Despite this, the appearance of nitrophytes on twigs prior to their appearance on trunks is consistent with the results of the extensive survey. Across the whole data set of the UK extensive survey, epiphytic twig communities have shown a better correlation with N atmospheric levels than trunk communities. The samples are more or less homogeneous in substrate age and not subject to as many environmental variables as the trunk. As we have shown in this comparison the trunk flora may respond to other environmental factors including shade, age of tree and ecological continuity.

The additional information on the recording sheet allows for some interpretation of the results e.g. increased bryophyte cover is usually associated with increasing shade, but in cases where epiphytic lichens are absent or diversity low, we need to know the range of environmental factors influencing lichen distribution. However, the trunk data may provide valuable historic information especially in sites where there is long ecological continuity, providing a comparison with epiphytic lichen communities established over a longer time period.

The appearance of nitrophytes in the twig flora may allow the identification of a threat from recent changes affecting the twig flora. This may be important in sites of conservation importance where BAP or other sensitive epiphytic species may be found on trunks. It may also indicate where changes in other elements of vegetation may be expected.

12.5. Conclusions

- The present study indicates that macrolichens on twigs and trunks could be used by non-experts as early indicators of N impacts at designated sites.

- Macrolichen indicator values are strongly negatively correlated with increasing NH_3 concentration using indicator values developed on the continent.

- Acidophyte and nitrophyte communities form a good basis for measuring changes in site condition in Britain, but indicator species need to be reassessed from data collected across the climatic gradient in the UK.

- Indicator lists based on species selected for a particular climate need to be evaluated when used in a range of conditions. This applies to van Herk lists and to the application of Ellenberg N values as both have been devised for conditions in continental Europe.

- In order to establish useful epiphytic indicators to detect changes in environmental conditions associated with N deposition, multivariate analysis of both lichen and environmental data needs to be carried out.

- Twig epiphytes show a better correlation with atmospheric N than trunk epiphytes but trunk epiphytes may carry indicators of previously conditions at the site.

13. Nitrogen impact assessment for 32 UK extensive sites: synthesis of biomonitoring methods

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13.1. Introduction

Impacts of atmospheric N are not explicitly assessed as part of the Common Standards Monitoring (CSM) assessments of designated statutory sites. One of the key objectives in the extensive UK study was to test, using conservation agency staff, the use of simplified N biomonitoring methods as early indicators of change and/or impact where elevated N inputs had been identified at a specific designated site. The simplified biomonitoring methods applied at each of the extensive UK study sites were: a) total N content of pleurocarpous mosses, b) soluble NH_4-N concentration of pleurocarpous moss species, c) macrolichen indicator species on trees and twigs and d) bark pH measurements.

The measurements from the different biomonitoring methodologies gave an indication of the epiphytic macrolichen indicator values, bark acidity and the current foliar N status of the selected individual moss species at individual sites.

At each of the UK sites, measurements of NH_3, SO_2 concentration and total N and S deposition inputs were obtained from current monitoring, historical monitoring, NEGTAP estimates and mapped data.

Using the gaseous NH_3 concentration and N and S deposition data and the results from the N biomonitoring methods, a generalised impact assessment of all 32 sites is provided in this section in relation to the habitat type of each site. This is a generalised assessment and CSM will not be considered in this study. It is essential that deployment of any N biomonitoring adds to the value of the existing monitoring strategies.

13.2. Site selection

The selection criteria for the 32 sites are fully described in Section 9. The sites in the UK extensive study are listed in Table 13.1 along with their principle habitat type, critical load and critical load exceedance. The NH_3 concentration data and the N and S inputs for the selected sites are provided in Tables 13.2 and 13.3.

Table 13.1. Description of sites in the UK Extensive study, indicating their habitat types and critical load exceedance**.**

Site no.	Site Name	Habitat type	Critical Load N deposition (kg N ha^{-1} y^{-1})	Exceedance of Critical Load
1	Cwmystwyth	Unimproved acid grassland	10 – 20	✓
2	Plynlimon	Upland heath	10 -20	
3	Dyffrn Mymbyr	Wet acid heath	10 – 20	✓
4	Stackpole	Lowland woodland	10 -15	✓
5	Eskdalemuir	Upland moorland	10 - 20	
6	Halladale	Improved grassland	10 - 20	
7	Strathvaich Dam	Upland moorland	10 - 20	
8	Glensaugh	Upland moorland	10 - 20	
9	Inverpolly/Knockan	Upland moorland	10 -20	
10	Edinburgh Centre	Parkland	10 -20	✓
11	Ariundle	Atlantic oak woodland	10 - 15	
12	Glen Nant	Atlantic oak woodland	10 - 15	✓
13	Wood of Cree	Atlantic oak woodland	10 - 15	✓
14	Bush	Parkland	10 - 15	✓
15	Sherwood (Ladybower)	Blanket mire+wet & dry heath	5 - 10	✓
16	Moorhouse	Blanket mire	5 - 10	✓
17	Fenns' Moss B	Raised bog	5 - 10	✓
18	Stanford	Grassland and heathland	10 - 20	✓
19	Fressingfield	Unimproved neutral grassland	20 - 30	✓
20	Bedlington/Bedingfield	Unimproved neutral grassland	20 -30	✓
21	Borrowdale	Upland oak woodland	10 - 15	✓
22	Brown Moss	Woodland and heathland	10 - 20	✓
23	Llnclys Common	Grassland, scrub and woodland	10 - 20	✓
24	Wytham Wood	Mixed broadleaved woodland & pasture	10 - 15	✓
25	Lullington Heath	Chalk heath and grassland	15 - 25	✓
26	Lough Navar	Forest	10 - 15	
27	Glenmore Wood	Mature acid oak woodland	10 - 15	✓
28	Caldanagh Bog	Lowland raised bog	5 - 10	✓
29	Castle Enigan	Lowalnd Fen	10 - 20	✓
30	Orritor	Oak woodland	10 - 20	✓
31	Redgrave and Lopham	Fenland	15 - 30	✓
32	Yarner Wood	Ancient oak woodland	10 - 15	✓

Table 13.2. The UK sites indicating their habitat types and NH_3 concentrations and annual mean SO_2 concentrations (where available).

Site no.	Site Name	Habitat type	NH_3 conc. ($\mu g\ m^{-3}$)	SO_2 concentration ($\mu g\ m^{-3}$)	SO_2 concentration ($\mu g\ m^{-3}$) Mapped values from APIS
1	Cwmystwyth	Unimproved acid grassland	2.96	1.11	1.5
2	Plynlimon	Upland heath	0.60		1.5
3	Dyffrn Mymbyr	Wet acid heath	1.18		2.6
4	Stackpole	Lowland woodland	1.81		2
5	Eskdalemuir	Upland moorland	0.38	0.82	1.1
6	Halladale	Improved grassland	0.83		1.2
7	Strathvaich Dam	Upland moorland	0.18	0.23	0.6
8	Glensaugh	Upland moorland	0.35	0.79	1.1
9	Inverpolly/Knockan	Upland moorland	0.12		0.8
10	Edinburgh Centre	Parkland	2.5		16.6
11	Ariundle	Atlantic oak woodland	0.04		1.4
12	Glen Nant	Atlantic oak woodland	0.06		1.6
13	Wood of Cree	Atlantic oak woodland	0.12		1.5
14	Bush	Parkland	0.9	1.81	3.7
15	Sherwood (Ladybower)	Blanket mire +wet & dry heath	0.73		5
16	Moorhouse	Blanket mire	0.44		2.2
17	Fenns' Moss B	Raised bog	1.87		2.2
18	Stanford	Grassland and heathland	1.54		2.8
19	Fressingfield	Unimproved neutral grassland	5.29		3.7
20	Bedlington/Bedingfield	Unimproved neutral grassland	7.43		3.8
21	Borrowdale	Upland oak woodland	0.19		2
22	Brown Moss	Woodland and heathland	3.95		2.7
23	Llnclys Common	Grassland, scrub and woodland	1.78		1.8
24	Wytham Wood	Mixed broadleaved wood & pasture	1.13		3.6
25	Lullington Heath	Chalk heath and grassland	0.9		3.3
26	Lough Navar	Forest	0.47	0.36	0.6
27	Glenmore Wood	Mature acid oak woodland	2.28		0.7
28	Caldanagh Bog	Lowland raised bog	2.58		1.5
29	Castle Enigan	Lowalnd Fen	6.45		2
30	Orritor	Oak woodland	4.09		3.9
31	Redgrave and Lopham	Fenland	2.28		3.7
32	Yarner Wood	Ancient oak woodland	0.65	1.05	1.1

Table 13.3. UK Extensive sites for general impact assessment using N biomonitoring methods. (The difference between the NH_3 deposition and total N deposition is the wet deposition plus NO_y dry deposition.)

Site no.	Site Name	NH_3 conc. $\mu g\ m^{-3}$	NH_3-N deposition (kg NH_3-N $ha^{-1}\ y^{-1}$)	Total N deposition (kg N $ha^{-1}\ y^{-1}$)	S deposition (kg S $ha^{-1}\ y^{-1}$)
1	Cwmystwyth	2.96	20.2	30.3	12.2
2	Plynlimon	0.6	4.2	13.6	13.3
3	Dyffrn Mymbyr	1.18	8.6	31.3	13.0
4	Stackpole	1.81	18.3	28.4	12.0
5	Eskdalemuir	0.38	2.3	14.2	10.4
6	Halladale	0.83	5.3	11.1	11.0
7	Strathvaich Dam	0.18	1.3	7.9	14.2
8	Glensaugh	0.35	2.0	12.4	7.7
9	Inverpolly/Knockan	0.12	0.8	5.4	9.6
10	Edinburgh Centre	2.5	15.5	26.5	21.9
11	Ariundle	0.04	0.4	11.3	22.24
12	Glen Nant	0.06	0.6	17.7	23.84
13	Wood of Cree	0.12	1.2	16.2	12.8
14	Bush	0.9	9.7	16.6	11.2
15	Sherwood (Ladybower)	0.73	2.7	31.2	24.6
16	Moorhouse	0.44	3.2	20.1	12.8
17	Fenns' Moss B	1.87	9.8	21.2	7.4
18	Stanford	1.54	8.0	22.3	9.0
19	Fressingfield	5.29	28	40.9	9.3
20	Bedlington/Bedingfield	7.43	39.9	53.4	9.4
21	Borrowdale	0.19	2.0	40.1	28.6
22	Brown Moss	3.95	21	32.8	8
23	Llnclys Common	1.78	9.2	20.5	7.4
24	Wytham Wood	1.13	10.7	33.1	8.3
25	Lullington Heath	0.9	4.7	14.8	10.0
26	Lough Navar	0.47	2.4	11.5	14.9
27	Glenmore Wood	2.28	12.5	32.5	13.8
28	Caldanagh Bog	2.58	13.9	22.1	8.3
29	Castle Enigan	4.09	21.9	32.7	10.1
30	Orritor	6.45	32.8	72.9	9.9
31	Redgrave and Lopham	2.28	12.0	25.2	10.4
32	Yarner Wood	0.65	4.1	31.2	18.2

13.3. Determination of mean habitat total N content for pleurocarpous moss species

In Section 10, a generalised tissue N content threshold value for pleurocarpous mosses was estimated by expert judgement to be 1.3% N. Therefore, UK extensive sites with tissue N contents above this threshold value of 1.3% N were considered to be impacted upon by atmospheric N inputs.

In this section an alternative approach using foliar concentrations (total tissue N and soluble NH_4-N concentrations) is described. By grouping the sites together by generalised habitat type, and using the data from all the moss species sampled at those sites, mean habitat total N concentrations can be determined for individual habitat types (Tables 13.4- 13.7) using the

data from all the moss species sampled at those sites. From the UK extensive sites, there were sufficient data to consider four main habitat types (upland moorland, lowland wetland, mixed broadleaved woodland and Atlantic oak woodland). Using the derived mean % N content for the specific habitat, an assessment of the habitat can be made based on foliar N concentration. There were differences between the mean total N content of the four habitats examined.

The upland habitats and the Atlantic oak woodland had the lowest mean N concentrations 1.10% N and 1.06% N, respectively, whilst the lowland wetland habitats were slightly higher at 1.30 % N with the mixed broadleaved woodland exhibiting 1.49% N. The mean N deposition values for the different habitats are; upland 12.3 kg N ha^{-1} y^{-1}; lowland wetland 26.8 kg N ha^{-1} y^{-1}; Atlantic oak woodland 21.3 kg N ha^{-1} y^{-1} and mixed broadleaved woodland 29.3 kg N ha^{-1} y^{-1}. The Atlantic oak woodland mean N deposition is larger than might be expected, due to the inclusion of Borrowdale, which has twice the N deposition of the other sites within the habitat type.

Although, these mean total N contents are based on a small dataset, there are detectable differences between habitats and this may provide a mechanism to help assess potential N impacts on sites with remote undefined diffuse N sources. Although, high foliar N contents might be expected at sites with a large wet N deposition, this has not always proved to be the case. In fact recent results have shown that high wet deposition of N does not usually result in high tissue N (C. Pitcairn pers. comm.), with the data (presented in Section 10) showing a stronger response of tissue N content to NH_3 concentrations and hence NH_3 dry deposition.

Table 13.4. The general impact assessment of upland habitat sites based on mean N content for these habitats of **1.10% N** content.

Site no.	Site	Total N content (% N dry weight)	Critical load exceedance	N impact based on mean foliar % N conc.	Impact assessment based on % N data
2	Plynlimon	1.11, 1.05, 1.02	No	None	Favourable
5	Eskdalemuir	0.94, 0.95	No	None	Favourable
7	Straithvaich	0.73, 0.78, 0.62	No	None	Favourable
8	Glensaugh	1.29, 1.31	No	Marginal	Unfavourable
9	Knockan	1.07, 1.63, 1.13	No	Marginal	Unfavourable
16	Moorhouse	1.27, 1.48	Yes	Yes	Unfavourabe

In using the mean N content criteria, the N contents for both Glensaugh and Knockan would indicate a slight increase in foliar N concentrations (Table 13.4), whereas Moorhouse would appear to be currently being impacted upon by N deposition. The N deposition at Moorhouse of 20.1 kg N ha^{-1} y^{-1} would indicate that total N deposition is above the critical load for this habitat and therefore the foliar N concentrations may be an early warning indicator of potential N impacts on the long-term integrity of this site.

Table 13.5. The general impact assessment of mixed broadleaved woodland habitats based on mean tissue N content for these habitats of **1.49% N** content.

Site no.	Site	Total N content (% N dry weight)	Critical load exceedance	N impact based on mean foliar % N conc.	Impact assessment based on % N data
23	Llyclys common	1.07, 1.08, 1.24	Yes	No	Favourable
24	Wytham Wood	0.96, 1.11	Yes	No	Favourable
27	Glenmore	1.87, 1.98, 1.62	No	Yes	Unfavourable
30	Orritor	1.95, 1.90, 2.12	Yes	Yes	Unfavourable
32	Yarner wood	1.30, 1.27	Yes	No	Favourable

The N contents at Orritor in Northern Ireland would strongly indicate that this site is currently being impacted by N deposition (Table 13.5). The N deposition of 72.9 kg N ha^{-1} y^{-1} for this site would confirm this, with approximately 50% of the total N deposition is NH_3-N derived indicating a large agricultural N background at this site. The current N deposition at Orritor could lead to possible long-term changes in species composition of non vascular plants. Glenmore also shows some indication of risk: although the critical load is not exceeded at present, the foliar % nitrogen values are above the critical value for this habitat, thus indicating a potential risk from N deposition.

Of the Atlantic oak woodland sites, only at Wood of Cree were foliar N contents measured, which could indicate potential N impact on this site (Table 13.6). In general, the N contents for the Atlantic oak woodlands are low and these sites are not considered at risk using the N content criteria. Although, the N deposition for Borrowdale is 41.1 kg N ha^{-1} y^{-1}, this high wet N deposition is not reflected in the foliar N contents for this site (Table 13.6). This is consistent with the findings of Pitcairn (pers comm.) that high wet N deposition does not always reflect high tissue N concentrations in upland species.

Table 13.6. The general impact assessment of Atlantic oak woodlands sites based on mean N content for these habitats of **1.06% N** content.

Site no.	Site	Total N content (% N dry weight)	Critical load exceedance	N impact based on mean foliar % N conc.	Impact assessment based on moss % N data
11	Ariundle	0.67, 0.91	No	No	Favourable
12	Glen Nant	1.04, 0.84, 0.79 0.72	Yes	No	Favourable
13	Wood of Cree	1.33, 1.76, 1.27	Yes	Yes	Possible long term impacts
21	Borrowdale	1.14, 1.06, 1.12	Yes	No	Favourable

The lowland wetland sites all exceeded their critical load (Table 13.1). Using the habitat-based approach, both Ladybower and Fenn's Moss did not indicate that there was an increase in N content of the mosses (Table 13.7). By contrast, the results suggest that both the Northern Ireland lowland wetland sites (Caldanagh Bog, Castle Enigan) are currently being impacted by N deposition and are potentially at long-term risk of changes in site integrity.

Table 13.7. The general impact assessment of lowland wetland habitat sites based on mean N content of **1.30% N.**

Site no.	Site	Total N content (% N dry weight)	Critical load exceedance	N impact based on mean foliar % N content.	Impact assessment based on moss % N data
15	Ladybower	0.99, 1.32	Yes	No	Favourable
17	Fenn's moss	0.92, 1.20	Yes	No	Favourable
28	Caldanagh Bog	1.56, 1.14, 1.56	Yes	yes	Unfavourable
29	Castle Enigan	1.59, 1.24, 1.49	Yes	yes	Unfavourable

13.4. Determination of mean habitat soluble NH_4-N concentration for pleurocarpous moss species

A mean habitat soluble NH_4-N concentration can be determined as in Section 10.3 for foliar % N by using the moss species data collected from the individual sites (Tables 13.8- 13.11). From the UK sites there were sufficient data to consider four main habitat types (upland moorland, lowland wetland, mixed broadleaved woodland and Atlantic oak woodland). Using the derived mean soluble NH_4-N concentration for the specific habitat, an assessment of the habitat can be made based on mean soluble NH_4-N concentration. There were differences between soluble NH_4-N concentrations of the four habitats examined. The upland habitats and the Atlantic oak woodland had the lowest mean N concentrations at 3.2 and 4.4 $\mu g\ g^{-1}$ FW respectively, whilst the lowland wetland habitats were higher at 6.8 $\mu g\ g^{-1}$ FW and the mixed broadleaved woodland at 7.7 $\mu g\ g^{-1}$ FW. The mean habitat N deposition for the different habitats are; upland 12.3 kg N $ha^{-1}\ y^{-1}$; lowland wetland 26.8 kg N $ha^{-1}\ y^{-1}$; Atlantic oak woodland 21.3 kg N $ha^{-1}\ y^{-1}$ and mixed woodland 29.3 kg N $ha^{-1}\ y^{-1}$.

Table 13.8. The general impact assessment of upland habitat sites based on mean soluble NH_4-N concentration for this habitat. (3.2 $\mu g\ g^{-1}$ FW).

Site no.	Site	Total NH_4-N concentration ($\mu g\ g^{-1}$ FW)	Critical load exceedance	N impact based on mean NH_4-N conc. ($\mu g\ g^{-1}$ FW)	Impact assessment based on NH_4-N concentration data
2	Plynlimon	1.6, 3.8, 2.6	No	No	Favourable
5	Eskdalemuir	1.4, 1.8	No	No	Favourable
7	Straithvaich	2.5, 0.9, 1.7	No	No	Favourable
8	Glensaugh	2.5, 1.8	No	Marginal	Marginal
9	Knockan	4.3, 9.4	No	Yes	Unfavourable
16	Moorhouse	5.1, 6.3	Yes	Yes	Unfavourable

Table 13.9. The general impact assessment of mixed broadleaved woodland habitats based on mean NH_4-N concentration (**7.7 μg g^{-1} FW**).

Site no.	Site	Total NH_4-N concentration (μg g^{-1} FW)	Critical load exceedance	N impact based on mean NH_4-N conc. (μg g^{-1} FW)	Impact assessment based on NH_4-N concentration data
23	Llyclys common	2.9, 2.9, 3.5	Yes	No	Favourable
24	Wytham Wood	2.9, 2.5	Yes	No	Favourable
27	Glenmore	13.8, 2.0, 15.1	No	Yes	Unfavourable
30	Orritor	21.7, 21.9	Yes	Yes	Unfavourable
32	Yarner wood	2.2, 1.2	No	No	Favourable

Table 13.10. The general impact assessment of Atlantic oak woodlands sites based on mean NH_4-N concentration (**4.4 μg g^{-1} FW**).

Site no.	Site	Total NH_4-N concentration (μg g^{-1} FW)	Critical load exceedance	N impact based on mean NH_4-N conc. (μg g^{-1} FW)	Impact assessment based on NH_4-N concentration data
11	Ariundle	2.5, 4.3	No	No	Favourable
12	Glen Nant	2.7, 1.3, 1.5, 4.0	No	No	Favourable
13	Wood of Cree	8.1, 8.9, 5.6	No	Yes	Unfavourable
21	Borrowdale	3.6, 4.8, 5.2	Yes	Yes	Marginal

Table 13.11. The general impact assessment of lowland wetland habitat sites based on mean NH_4-N concentration (**6.8 μg g^{-1} FW**).

Site no.	Site	Total NH_4-N concentration (μg g^{-1} FW)	Critical load exceedance	N impact based on mean NH_4-N conc. (μg g^{-1} FW)	Impact assessment based on NH_4-N concentration data
15	Ladybower	2.6, 4.2	Yes	No	Favourable
17	Fenn's moss	3.3, 2.8	Yes	No	Favourable
28	Caldanagh Bog	10.4, 9.5, 10.6	Yes	Yes	Unfavourable
29	Castle Enigan	12.9, 3.9, 7.9	Yes	Yes	Unfavourable

Fourteen out of the 32 UK Extensive sites had pleurocarpous moss species had soluble NH_4-N concentrations > than the set general threshold value of 6 μg g^{-1} FW, whereas, 23 out of the 32 sites had critical load exceedance. This result suggests that the critical load method could be overestimating the number of sites at risk form N impacts. It is interest to note that there were species differences in soluble NH_4-N concentration with *R. squarrosus*, *E. praelongum*, *E. striatum* and *B. rutabulum* having consistently higher concentrations. This could be directly related to their habitat preferences and presence at sites with higher N deposition.

13.5. Estimate of N deposition using pleurocarpous moss total N content data

Sutton *et al.* (2004a) applied the relationship between foliar nitrogen content of pleurocarpous mosses, from variety of diffuse and point source attribute sites throughout the UK, and N deposition to estimate the N deposition from a site based on the foliar N content. This simple power function relationship was used to estimate the N deposition for the foliar N content in the pleurocarpous mosses sampled from the UK extensive study. These estimated foliar N content derived N deposition were then plotted against measured N deposition (kg N ha^{-1} y^{-1}) to determine the robustness of the method when plotted against known N deposition.

The results show that there was a very weak relationship between estimated N deposition using the tissue N content data from the UK extensive sites and the measured N deposition (Figure 13.1). The N content data in the current study were from sites diffuse sources, whereas, the data used in the Phase 1 Report (Sutton *et al*. 2004a) was a combination of point and diffuse source sites. This difference could have contributed to the poor relationship as it has been reported that dry N deposition will have a greater effect on foliar N content of species than wet N deposition on a per unit N basis (Leith *et al*. 2001).

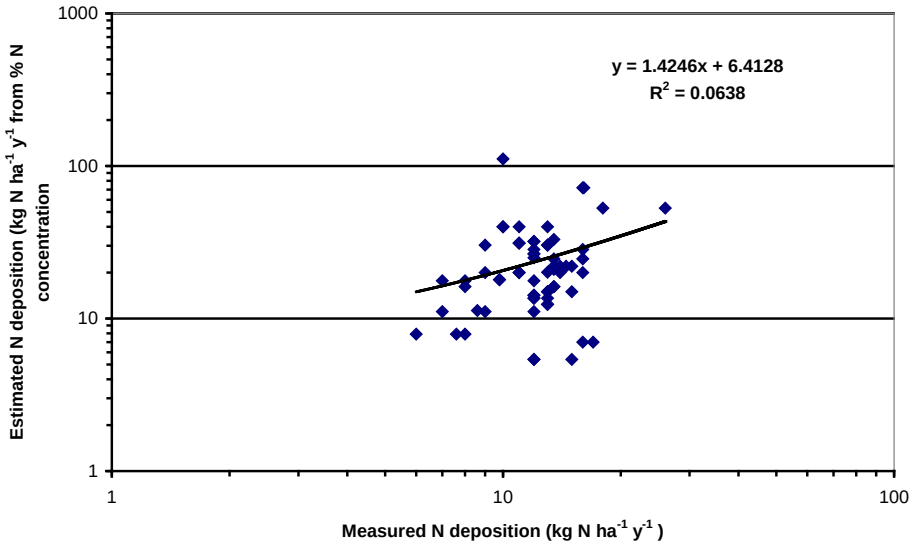

Figure 13.1. Plot showing no relationship between estimated N deposition using pleurocarpous moss total N contents and measured N deposition.

13.6. Lichen indicator values

Macrolichens sampled on trunks and twigs of available trees in all sites were used to calculate acidophyte, nitrophyte and Ellenberg-N values at all sites. Although acid-barked deciduous trees were recommended for sampling, 8 sites had either trees with a high bark pH or non-native crop trees (e.g. sitka spruce at Plynlimon), which were unsuitable for this kind of study. In addition *Betula* species were sampled on five sites. Although twigs of *Betula* spp. present a good substrate for epiphytic lichens the peeling bark of trunks makes this a less suitable tree for epiphytes on trunks. Sites that contained less suitable/unsuitable tree species are distinguished with * in Table 13.12. If sites are situated on unsuitable substrates then the absence of acidophytes or nitrophytes may be due to a number of these factors and must be considered in the light of other factors such as rainfall and occult precipitation, available tree species and bark pH and available propagules.

If nitrophytes are present on twigs there is assumed to be some risk, if present on trunks and twigs on acid barked trees there is already an established source of nitrogen. When this is combined with low values for acidophytes on acid-barked trees there is further evidence of a response. However, when basic-barked trees such as *Populus nigra*, *Fraxinus* and *Acer* species are sampled, it is not easy to interpret the results until additional evidence is obtained. In regions of high rainfall the loss of acidophytes and the appearance of nitrophytes may be delayed. In these sites appearance of nitrophytes is more critical than in regions of rainfall <1000 mm. Figure 13.2 shows a risk assessment of sites and where possible the sites have been assessed in Table 13.12.

Table 13.12. Table of sites sampled on extensive lichen survey including: tree species, habitat indicated as upland (U), deciduous woodland (D), lowland central UK woodland (DP) Atlantic woodlands (AD) and wetlands (W), with summaries of lichen acidophyte (A) and nitrophyte (N) and lichen Ellenberg_N (Ell) results on trunks (tr) and on twigs (tw). All lichen results with * are from non-oak substrata. Areas of high rainfall are distinguished (Y) and threat level from nitrogen given where possible.

Site no.	Site name	Tree spp.	Habitat	CL	Acidophyte summary	Nitrophyte summary	Ellenberg N summary	Condition	High rainfall	Threat level from N
1	Cymystwyth	Qu		X	A <15 on tr, A <5 on tw	N absent	Ell <5 on tr & tw	Good no nitrophytes	Y	minimal
2	Plynlimon	Ss	U		A* >10 on tr	N absent	Ell <5 on tr	unsuitable substrate		
3	Dyffryn Mwmbyr	Ap		X	A*<5 on tr	N absent	Ell <5 on tr	no N, low div but probably good		minimal
4	Stackpole	Qu	D	X	A absent on tr A >5 on tw	N absent on tr A <5 on tw	Ell 0 on tr, <10 on tw	Some change due to N on twigs	Y	some
5	Eskdalemuir Observatory	Pn	D		A* >5 on tr, A <5 on tw	N absent on tr, N <1 on tw	Ell >10 on tr, <5 on tw	N absent + high bark pH	Y	minimal*
6	Halladale	Bp	U		A* >5 on Tr & Tw	N absent	Ell >5 on tr,<5 on tw	Good	Y	none
7	Strathvaich Dam	Bp	U		A* >15 on Tr, <1 on tw	N absent	Ell 15 on tr, <5 on tw	Good	Y	none
8	Glensaugh	Sa	U		A* absent on tr and<5 on tw	N <1 on Tr & 1 onTw	Ell >5 on tr <5 on tw	Good but nitrophytes appearing on twigs		some
9	Knochan (Inverpolly)	Sa	D	X	A* >5 on tr & absent on tw	N absent	Ell & div low	previous acid rain	Y	
10	Edinburgh centre	Fx	AD	X	A >5 on tr & tw	N absent on tr, 1 on tw	Ell high on twigs	good but nitrophytes appearing on twigs		some

Site no.	Site name	Tree spp.	Habitat	CL	Acidophyte summary	Nitrophyte summary	Ellenberg N summary	Condition	High rainfall	Threat level from N
11	Ariundle	Qu			A<5 on tr, >5 on tw	N <5 on tr & tw	Ell high on twigs	not good…. Nitrophytes present on trunks and twigs	Y	high
13	Wood of Cree	Qu	AD	X	A >20 on tr, <1 on tw	N >5 on tr, 0 on tw	Ell <20 on tr, 0 on tw	nitrophytes present	Y	moderate
14	Bush	Qu	D	X	A >10 on tr,<1 on tw	N 0 on tr, <1 on tw	Ell 10 on tr, >5 on tw	nitrophytes present on twigs		moderate
15	Ladybower	Qu	W	X	A <5 on tr, 0 on tw	N absent	Ell<5 on tr & tw	low values for A and Ell suggest former acidification	Y	none
16	Moorhouse	Bp	U	X	A* >15 on tr, <1 on tw	N absent	Ell >5 on tr & tw	nitrophytes absent	Y	*
17	Fenns moss b	Le	W	X	A absent on tr, <1 on tw	N absent	Ell <1 on tr, >10 on tw	unsuitable substrate but no nitrophytes		*
18	Stanford	Qu	DP	X	A absent on tr & <5 on tw	N absent on tr, <5 on tw	Ell <5 on tr & <20 on tw	not good, post SO_x and N sp not arrived		none
19	Fressing field	Qu	DP	X	A <5 on tr, absent on tw	N <15 on tr, >5 on tw	Ell >30 on tr,>10 on tw	very bad		high
20	Bedlingfield	Qu	DP	X	A <10 on tr, <1 on tw	N absent on tr, <1 on tw	Ell <5 on tr & tw	not good…. N on trunks and twigs, A disappearing		high
21	Borrowdale	Qu	AD	X	A absent on tr & tw	N 5 on tr, 10 on tw	Ell <10 on tr ,>10 on tw	bad, acidophytes absent on acid bark, N high on twigs	Y	high
22	Brown moss	Qu	DP	X	A absent on tr & tw	N <5 on tr & tw	Ell <10 on tr & >10 on tw	bad, acidophytes absent on acid bark, N high on twigs		high
23	Llynclys common	Qu	D	X	A absent on tr & tw	N <5 on tr & tw	Ell <5 on tr & tw	bad, acidophytes absent on acid bark		high

Site no.	Site name	Tree spp.	Habitat	CL	Acidophyte summary	Nitrophyte summary	Ellenberg N summary	Condition	High rainfall	Threat level from N
24	Wytham wood	Qu	D	X	A absent on tr <5 on tw	N absent on tr, <5 on tw	Ell <5 on tr ,>10 on tw	acidophytes absent but N appearing on twigs		moderate
25	Lullington heath	Cm	DP	X	A* absent on tr, >15 on tw	N absent on tr,<5 on tw	Ell >5 on tr, >15 on tw	good site but nitrophytes appearing on twigs		moderate
26	Lough navar	Bp			A* >15 on Tr, <1 on tw	N absent on tr,<5 on tw	Ell >5 on tr & <5 ontw	A disappearing on tw and N appearing, div low	Y	moderate
27	Glenmore wood	Qu	D	X	A absent on tr & tw	N absent on tr, <5 on tw	Ell <5 on tr, >5 on tw	acidophytes absent and N presnt on twigs … something happening		high
28	Caldanagh bog	Fx	W	X	A*absent on tr & tw	N <5 on tr & <10 on tw	Ell <5 on tr, >10 on tw	not good…. Nitrophytes present on trunks and twigs		high
29	Castle Enigan	Cm	W	X	A* absent on tr & tw	N absent on tr, >5 on tw	Ell 0 on tr, >15 on tw	not good		high
30	Orritor	Fx	D	X	A* absent	N >5 on tw	Ell >15 on tw	not good	Y	high
32	Yarner Wood	Qu	*D*	X	A >10 on tr & tw	N absent on tr, <5 on tw	Ell <10 on tr, 10 on tw	A still high on tr and tw but N appearing on twigs	Y	moderate

Key to the tree species: Qu: *Quercus*; Bp: *Betula* spp.; Fx: *Fraxinus*; Cm: *Crateagus monogyna*; SS; *Sitka* spruce (See Section 11.2.2).

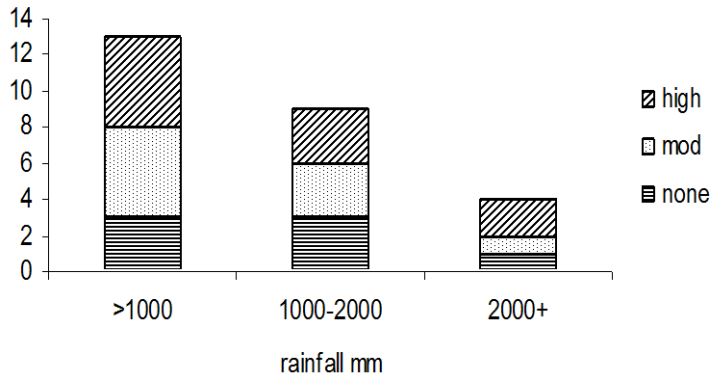

Figure 13.2. Distribution of sites under varying degrees of threat as categories using lichen information in table 13.12 by rainfall groups.

The appearance of nitrophytes in oceanic sites exhibiting high rainfall volumes, such at Ariundle and Wood of Cree, may not be due to local sources of N but may be due to long distance transport forms of nitrogen such as ammonium (van Herk 2003). In this situation, monitoring of acidophytes on twigs and trunks can be used to detect loss of acidophytes and detect early appearance of /or an increase in nitrophytes.

13.7. Comparison of UK extensive pleurocarpous moss tissue N contents and soluble NH_4-N concentrations with annual rainfall

The foliar N content and the soluble NH_4-N concentrations for the pleurocarpous mosses sampled in the UK study were plotted against the 5 year annual mean rainfall for the individual sites using Meteorological Office 5 x 5 km data (Figures 13.3 and 13.4). The results would suggest that annual rainfall influences N content and to a lesser extent soluble NH_4-N concentration. As precipitation increases there appears to be a trend for the total foliar N content in pleurocarpous mosses to decrease. Therefore, this result indicates the importance of taking into account local climatic factors when considering the data from the UK as a whole and emphasises the probable contribution of site characteristics to poor relationships between foliar N content and N deposition on a UK scale (as illustrated in Section 10; Figure 10.9). There appear to be species differences in % N content in response to rain volume, for example *R. triquetrus* % N contents does not appear to be affected by precipitation volume. Whereas, *T. tamariscinum* at higher precipitation sites (2500 mm) tends to have a lower tissue N content (~1.0% N) whilst at lower precipitation sites (1000-1500 mm) the % N has increased to 1.6-2.1% N. On the other hand *R. loreus* appears to favour the wetter habitats and consequently has lower % N contents.

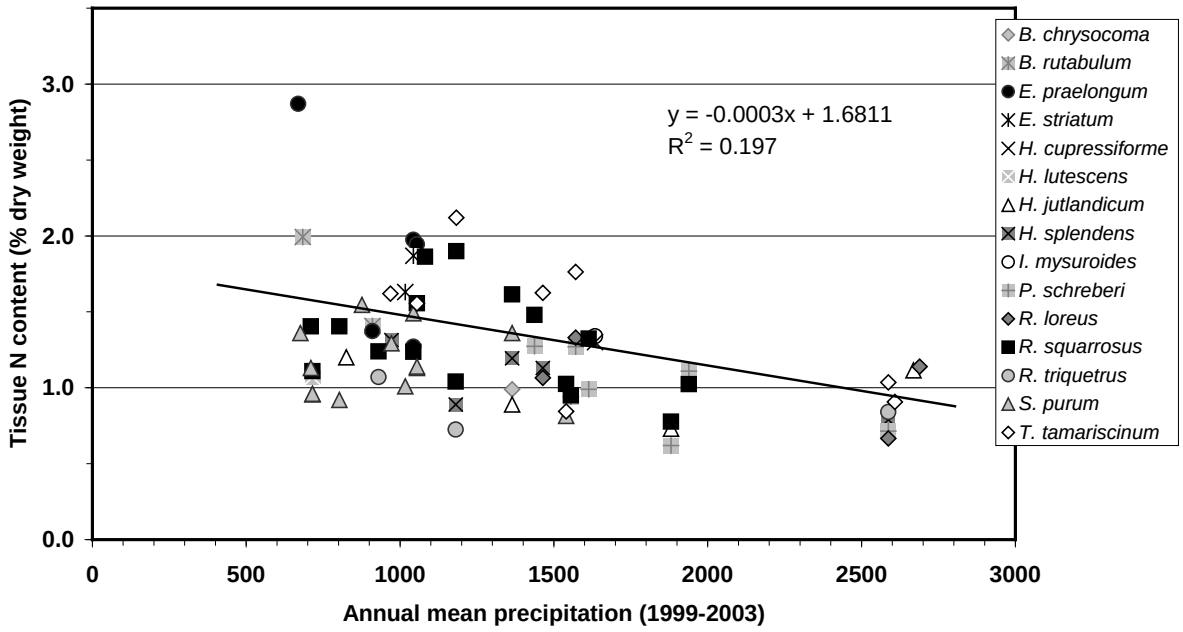

Figure 13.3. The total tissue N content of pleurocarpous mosses collected from UK Extensive sites plotted against the 5-year annual mean precipitation (5-year annual mean precipitation using 5 x5 km data).

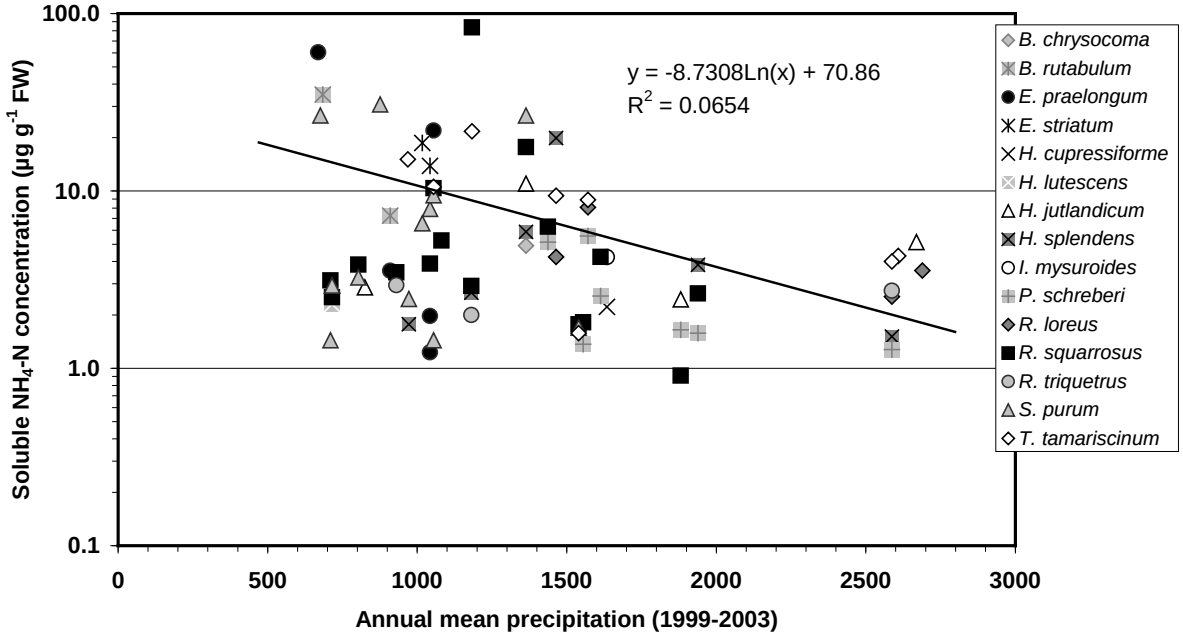

Figure 13.4. The soluble NH_4-N concentration data for the UK Extensive site pleurocarpous mosses plotted against the 5- year annual mean precipitation (5-year annual mean precipitation using Meteorological Office 5 x5 km data).

13.8. Conclusions

- The application of simplified biomonitoring on a UK scale using local field officers to conduct a lichen survey, collect moss, twig and bark samples worked well. Using the field officers allowed a greater number and range of habitats to be sampled.

- Annual rainfall appears to influence the N content of pleurocarpous mosses with a trend for increased precipitation reducing the foliar N content in the mosses across the UK Extensive sites. This is a general trend with a number of other potential factors influencing the relationship.

- *T. tamariscinum* has a lower N content (1% N) at higher precipitation sites (2500 mm) compared to a higher N content (1.6-2.1% N) at sites with a lower annual precipitation (1000-1500 mm).

- Moss foliar N concentration data shows a relationship with 5 year mean precipitation data (5 x 5 km^2). This indicates that it is important to take into account local climatic factors when considering the impacts of N. In addition, the data presented in Section 13.7 indicate an interactive effect with rain and N deposition on the foliar N results.

- Mean habitat foliar N and soluble NH_4-N concentrations were derived for four habitat types. The results show that it is possible to determine a distinguishable mean concentration for the different habitat types using the moss data collected as part of the UK extensive study.

- The upland moorland and Atlantic oak woodland had the lowest foliar total N content (106 and 1.10% N respectively) followed by the lowland wetlands at 1.30% N and finally the mature woodland at 1.45% N. A similar ranking in soluble NH_4-N concentrations was found for these habitats. However, there was virtually no difference between the lowland wetland and the mature woodland 7.4 and 7.7 $\mu g\ g^{-1}$ FW respectively.

- Macrolichen indicators on acid-barked trees show a decrease in acidophyte values and an increase in nitrophyte values with increasing ammonia concentration on both trunks and twigs, and to a lesser extent with total N deposition.

- The use of lichen indicators on twigs and trunks provides for a more robust assessment since due to the different bark pH of twigs and trunks it allows estimates to be made using more than one lichen community type. The increased sensitivity of twig lichens to ammonia compared with trunks can be attributed to the higher natural pH of twigs in comparison with trunk pH. In addition, such comparison between twigs and trunks has the potential to assess the change in conditions over time.

14. Biomonitoring protocols for example scenarios

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14.1. Introduction

Currently, assessment of N impacts on condition and long-term integrity of designated sites is not part of Condition Standards Monitoring (CSM) (See Section 15.1). If the conservation agencies are to apply biomonitoring methods at specific sites, identified as being potentially impacted by N inputs, it is necessary to have a set of protocols, which could be applied for a range of N input types and a wide range of habitats. The protocols presented below have been tailored to the N input source (i.e. direct gaseous NH_3 at a point source or diffuse wet N deposition) and not at any specific habitat type.

The objectives of the current study were to produce biomonitoring protocols, which could be applied by conservation and environment agency staff at generic habitats with different N sources. The scenarios needed to reflect different situations to which the agencies are likely to apply nitrogen biomonitoring methods. As each situation will be different, a generic approach is necessary. However, factors will be identified within each scenario, which have specific importance.

Four scenarios were identified by the Project Steering Group for assessment of the applicability of biomonitoring methods:

1. Assessment of N effects on plant communities in the vicinity of a large poultry farm (NH_3 dominated),
2. Assessment of the impacts of a large industrial point source close to a SAC (NO_x dominated),
3. Assessment of the effects on site condition/integrity from long range N deposition inputs on an upland site,

and

4. Assessment of the impacts on a lowland site with critical load exceedance, but no N point source. This scenario is particularly important as a number of the sites used in the UK extensive study (Section 9) would be in this category.

These monitoring protocols could include an integrated approach of using physical/chemical monitoring of nitrogen inputs as well as one or more bio-monitoring methods fit for the particular application.

It should be noted that the scenarios 1 and 2 could to be applied in the impact assessment required under the Habitats Regulations/PPC Regulations as part of an application for a PPC permit. Such requirements could include environmental monitoring to assess potential impacts directly associated with the regulated installation.

14.2. Scenarios

14.2.1. Scenario 1. Assessing the impacts of a large poultry farm on a lowland SAC

This scenario covers situations where a SAC is adjacent to a farm unit with a defined N source. The objective of monitoring in such a scenario is to assess current impacts of the farm on the site in relation to site integrity (i.e. effects) and to provide greater confidence in the estimates of concentrations/deposition of NH_3 to the site (i.e. exposure) (Table 14.1).

With a defined N pollution gradient away from an intensive farm unit, a transect (50-100 m upwind to 500-600 m downwind of the farm unit) can be established with defined sampling points that also ideally include physical monitoring of atmospheric NH_3 concentrations. These points should be distributed exponentially at distances (m) from the farm. The length of the transect is dependent on the uniformity of the habitat, the size of the NH_3 source and the land use downwind of the farm but should be at least 250-600 m in length in order to have a control point with background NH_3 concentrations, which are representative of the surrounding area.

An additional scenario with the SAC not adjacent to the farm unit but situated between 200-500 m from the NH_3 source will also be considered in this section. This case is much harder to attribute impacts to the N source, as the gradient in gaseous NH_3 concentration will not be as strongly defined as the situation with the SAC adjacent to the agricultural source.

The results from the current study have established that there are simplified biomonitoring methods that could be applied to assess the current N impacts and also monitor the integrity of a SAC close to a poultry farm. The biomonitoring method applied would be dependent on habitat type and the interest features of the SAC. The advantage of biomonitoring over sole reliance on physical monitoring is that it will identify changes/effects/integrity on the vegetation associated with the SAC vegetation in relation to a known N input, whereas physical monitoring alone can only identify that there is potential for N impacting.

Table 14.1. Preliminary decision tree for selection of nitrogen bioindicator methods for an agricultural point source (poultry farm) at a lowland SAC.

1: To assess effects of poultry farm on lowland SAC.	
	a. **Assess the impacts** of enhanced N concentrations or deposition? *Go to 2.*
	b. **Assess the magnitude** of atmospheric N concentrations or deposition? *Go to 7*
2: Obvious local source present: Visual assessment of the site and odours (e.g. livestock smell) may be sufficient. Use local wind rose data if this is available. In the case of point or line sources, measurements using bioindicators should focus on differences with distance from the source.	
Set up a transect extending from 100 m upwind to 600 m downwind of farm. The downwind distance will be dictated by the uniformity of habitat along the NH_3 gradient. The important criterion is that the furthest point along the gradient represents the background ambient concentration for the surrounding area. Atmospheric monitoring may be necessary depending if site has current monitoring or has had recently. As wind can blow from any direction it is important for the upwind site to be at a sufficient distant to give a background NH_3 concentration which is representative of the surrounding area i.e. at least 100 m.	
3: Make *a species list* *of higher plants and bryophyte species* in the ground flora.	
Determine *mean Ellenberg N Index of higher plants and bryophyte species* for at least 5 positions along transect. (Note: Interpretation of the method may be complicated by local differences of habitat management and soil types, and is therefore best suited to application at sites with well defined site history.)	

4: Site wooded or trees present? *Go to 5*

Site treeless. *Go to 6.*

5: Simplified macrolichen survey of epiphytic species on twigs and trunks using revised indicator species for UK conducted by trained non-experts. *Method: Survey of key lichen species and calculation of acidophyte/nitrophyte scores.* (Notes: The method gives a broad indication of NH_3 exposure). Where possible sampling is done on acid-barked tree species on trunks and twigs in order to compare present conditions for lichen establishment on new substrata with established lichen communities on older substrata (See Appendix III for details of macrolichen sampling protocols).

6: Standardised response to nitrogen deposition. *Method: Standardised grass plants (Lolium or Deschampsia) (*see Section 5 and Appendix II*).* (Note: the method is good for visual demonstration and directly records additional nitrogen absorption by the plants. For long-term application, a series of plants must be exposed over time).

7: Trees absent *Go to 8*

Trees present *Go to 9*

Atmospheric monitoring *Go to 10*

8: Foliar responses to different forms of additional nitrogen. (Note that the dose response relationships probably differ for different N forms).

Total tissue N of foliage. (Note: suited to pleurocarpous mosses and certain well studied higher plants, e.g. *Calluna vulgaris*). A well-studied parameter.

Soluble ammonium concentration of foliage. (Note: A newly identified parameter, which appears to show a very large response to enhanced N). Particularly large responses for pleurocarpous mosses (See section 8 & 10).

9: If trees present you can do simplified macrolichen frequency (See number 5 for details)

10: Atmospheric NH_3 concentrations: Passive NH_3 samplers (ALPHA) available for low cost atmospheric monitoring. The other N pollutants deposition can be obtained from national background maps (See Piddles Wood deposition data for example).

In cases where the SAC is not adjacent to the agricultural point source but in the vicinity (i.e.200-600 m downwind from the source) the above biomonitoring decision tree could still be applicable. However, due to potentially lower N concentration gradient/deposition the attribution of impacts on the vegetation will be harder to determine, especially in the short term. In such cases air dispersion modelling and physical atmospheric monitoring may be initially more appropriate. Depending on the outcome of these, longer-term biomonitoring could be applied. The single or combined biomonitoring method applied will depend on the NH_3 concentration gradient and the type and uniformity of habitat. The habitat type is important as gaseous NH_3 dispersion and deposition processes are dependent on the habitat structure as well as meteorological conditions i.e. NH_3 is an extremely reactive gas with deposition to surfaces greatest close to the source and when surfaces are wet.

Ellenberg N Index would indicate any longer term changes resulting from the pollutant source. If trees were present the use of the simplified acidophyte/nitrpohyte macrolichen method would indicate any change in species frequency and composition which would be an ‘early indication’ of impact.

In general, this scenario where the agricultural source is not adjacent to the lowland SAC is much more difficult for the conservation and environment agencies to legislate against as the critical load may not be exceeded at these sites, particularly as the agencies place great emphasis on the critical load values when determining permit conditions. It is important for site officers when assessing a site, to identify the pollutant source (or proportionate sources) and strength, its position in relation to the SAC (i.e. a SAC down-wind of the prevailing wind direction will be at

a greater risk than an upwind site) and the potential short and long term impacts to the interest feature.

Depending on the above, the use of biomonitors to determine short-term change may be inappropriate. However, the use of a combination of physical monitoring and longer-term biomonitoring may be the most appropriate approach to provide an early warning signal of the potential impacts to SACs in the vicinity of agricultural point sources.

14.2.2. Scenario 2. Assessing the impacts of a large industrial point source on a local SAC

This scenario assesses the impacts or potential impacts associated with NO_x emissions from a known point source on a local SAC. Unlike the agricultural point source (Section 14.2.1), emissions of NO_x from point sources generally do not lead to very high local concentrations or deposition. The determination of local NO_x concentrations from a single point source can also be complicated by the type or combination of other local N pollutants emitted and their concentrations i.e. vehicle emissions. NO_x emitted from a stack (depending on the height of the stack) could impact locally or ground at some distance from the stack with regional scale implications. Therefore, a primary assessment to identify the areas most at risk (i.e. position of maximum ground level NO_x concentration) from the source is essential. Depending on the concentration and habitat, this primary assessment will determine if biomonitoring could add to the overall assessment using air dispersion modelling and physical monitoring. It is always important to have physical monitoring or a historical record of the pollutants emitted. It would also be useful for assessment of current status/condition and also for long term integrity to have habitat species composition data, foliar N concentrations, or physical monitoring of the SAC prior to the industrial plant being established.

Table 14.2. Preliminary decision tree for selection of nitrogen bioindicator methods to assess impacts of a large industrial source on a SAC.

1: To assess effects of large industrial point source on local SAC
a. **Assess the impacts** of enhanced N concentrations or deposition? *Go to 2.* b. **Assess the magnitude** of atmospheric N concentrations or deposition? *Go to 7*
2: Obvious local source present: Visual assessment of the site and odours. As the installation is a known emitter of NO_x and other pollutants, obtain emission data from either the installation or through the Pollution Release Inventory (e.g. SEPA are custodians of the Scottish Inventory), any ecological impact assessments done as part of an EIA prior to the plant being established and obtain previous condition assessment of SAC. Use air dispersion modelling to identify areas that are potentially most at risk from NO_X emission source. Having established areas at risk, set up transect extending from 100 m upwind to 500 m downwind of and around the perimeter of the source making use of wind rose data etc which should be provided. The length of the transect will depend on the habitat uniformity and the emission size of the installation. Take account of any additional sources in the area such as roads and other industrial plants. If possible find a 'clean' area with a similar habitat for a 'normal' comparison. **3: Make a species list** of higher plants and bryophyte *species* present immediately around the source and in the ground flora. Determine *mean Ellenberg N Index of higher plants and bryophyte species* for at least 5 positions along transect. (Note: Interpretation of the method may be complicated by local differences of habitat management and soil types, and is therefore best suited to application at sites with a well defined site history) along the transect. Ellenberg Index could indicate enhanced N deposition at the SAC.

4: If site wooded or trees present? *Go to 5*

Site treeless. *Go to 6.*

5: Simplified macrolichen survey of epiphytic species on twigs and trunks using revised indicator species for UK conducted by trained non-experts. *Method: Survey of key lichen species and calculation of acidophyte/nitrophyte scores.* This method is more suited to detecting NH_3 impacts but may also provide some evidence of acidification resulting from NO_x exposure and if sufficient time standardised grass response can be included.

6: Standardised response to N deposition. Suitable if area is vandal free. Method: Standardised grass plants *(Lolium or Deschampsia)*. (Note: the method is good for visual demonstration and directly records additional nitrogen absorption by the plants. For long-term application, a series of plants must be exposed over time.) The suitability of this longer-term approach will be dependent on the source strength.

7: Trees absent *Go to 8*

Trees present *Go to 9*

Atmospheric monitoring *Go to 10*

8: Foliar responses to additional nitrogen and other pollutants.

Total tissue N of foliage. (Note: Suited to pleurocarpous mosses and certain well studied higher plants, e.g. *Calluna*). Important to identify the interest feature and if possible use the species listed.

Total tissue content of sulphur (S) and heavy metals if presence is suspected.

Soluble ammonium content of foliage. (Note: A newly identified parameter, which appears to show a very large response to enhanced N). Particularly large responses for pleurocarpous mosses.

Apply low cost methods for physical measurement of NO_x concentrations (e.g. diffusion samplers) in combination with above.

9: If trees are present a simplified macrolichen frequency can be performed.

10: Atmospheric NO_x concentrations

There is no bioindicator method currently available to indicate NO_x concentrations. Methods may yet be developed using lichens, but apart from near NO_x point or line sources responses appear to be stronger to NH_3. The established biomonitoring methods for NH_3 would give an indication of impact on and status of the site (depending on point source strength for NO_X and presence of other sources) but it would be more difficult to directly attribute these to a specific NO_x emission source.

Apply low cost methods for physical measurement of NO_x concentrations (e.g. diffusion samplers)

This scenario is much more complex than the agricultural one for conservation and environment agencies to legislate for (Section 14.2.1.). Biomonitoring methods can easily be applied to the agriculture scenario without the need for air dispersion modelling, as there is a defined localised gradient of NH_3 concentration from a known point source with the NH_3 concentrations being determined by physical monitoring along the concentration gradient. With NO_x it could be difficult to define a gradient of concentration or whether the critical load is being exceeded for the targeted SAC (depending on the distance to maximum ground concentration, other localised sources of the N within the proximity of the SAC and habitat type). These factors make the use of biomonitors much less robust for attributing impacts to NO_x sources.

Short-term NO_x impacts on the interest features of the SAC could be difficult to measure. However, if biomonitoring was required, the use of the sensitive macrolichen method could be used as an early indication of N impact to the SAC.

The combination of physical monitoring, modelling and selected biomonitoring method/s may be the most appropriate approach for sites with a NO_x source to give an indication of impacts on the site and its long term integrity.

14.2.3. Scenario 3. Assessing the impacts of long-range wet dominated N inputs at an upland SAC

The assessment of N impacts on remote upland areas with a diffuse source of N deposition is more complex than for the SAC's with a defined point source as in scenarios 1 and 2. The N deposition to these sites may be relatively small but continued N accumulation over a longer period could lead to changes in integrity. Upland vegetation is also adapted to low N availability and would be sensitive to large increases in available N through increased wet N deposition (as either reduced or oxidised N). It is important to note that other factors such as management practices and climatic changes could also be impacting on the SAC and causing potential changes in integrity, which are independent of N deposition but could make it difficult to determine if observed changes are due to N deposition or other factors (climatic, management practices, grazing pressures) or a combination of the two.

Table 14.3. Preliminary decision tree for selection of nitrogen bioindicator methods for upland SAC sites.

1: To assess impacts of long-range N inputs at an upland SAC site

a. **Assess the impacts** of enhanced N concentrations or deposition? *Go to 2.*
b. **Assess the magnitude** of atmospheric N concentrations or deposition? *Go to 7*

2: Obvious local source present: There is unlikely to be any local point sources at this type of site. The source will probably be long-range dominated by diffuse wet N deposition. Enhanced N inputs from occult precipitation may be important depending on the altitude of the site i.e. increasing ionic concentrations in precipitation by at least a factor of two. If livestock are present, check stocking rates for local variation in N deposition. Determine N input for habitat from sources such as APIS.

Set up a number of replicate quadrats around the site for long term integrity/ species composition change monitoring.

3: Make *a species list* *of higher plants and bryophyte species* in the ground flora.

A species list of the site is important for long-term assessment of integrity changes but the use of the Ellenberg N Index is unlikely to give a strong indication of N impact at these diffuse N sites. If Ellenberg Index is applicable then determine the *mean Ellenberg N Index of higher plants and bryophyte species* for at least 5 quadrats at the site (Note: Interpretation of the method may be complicated by local differences of habitat management and soil types, and is therefore best suited to application at sites with well defined site history). This survey method would be beneficial as part of a temporal assessment, or wider spatial survey and not as a one off assessment. The use of the simplified acidophyte/nitrophyte method could also be a useful initial assessment.

4: Site wooded or trees present? *Go to 5*

Site treeless. *Go to 6.*

5: Simplified macrolichen survey of epiphytic species on twigs and trunks using revised indicator species for UK conducted by trained non-experts. *Method: Survey of key lichen species and calculation of Acidophyte / Nitrophyte scores.* (Notes: The method gives a broad indication of NH_3 exposure. Where possible sampling is done on acid-barked tree species on trunks and twigs in order to compare present conditions for lichen establishment on new substrata with established lichen communities on older substrata. See also Bark pH.

6: Standardised response to N deposition. *Method: Standardised grass plants (Lolium or Deschampsia).* (Note: the method is good for visual demonstration and directly records additional N absorption by the plants). For long-term application, a series of plants must be exposed over time. As the N inputs will be low, the use of standardised grass plants will require long-term

exposure (6-12 months) and modified protocols to assess N impacts. The use of other slow growing species such as *Agrostis canina* and *Nardus stricta* could be considered but these would require further field testing. The selection of species is dependent on the habitat type. The ideal situation would be if the selected species was occurring naturally at the selected site. This method is not suitable for use in determining long term integrity of a site.

7: Trees absent *Go to 8*

Trees present *Go to 9*

Atmospheric monitoring *Go to 10*

8: Foliar responses to different forms of additional nitrogen. (Note that the dose response relationships probably differ for different N forms).

Total tissue N of foliage. (Note: suited to pleurocarpous mosses and certain well studied higher plants, e.g. *Calluna vulgaris*). The use of mosses and *C. vulgaris* are well-studied pollution biomonitoring parameters. Tissue N in moss species could be more related to concentration in the precipitation than N deposition. Due to possible seasonal variations in foliar N concentration and the importance of the hydration status of pleurocarpous mosses sampling should be carried out in late spring or early autumn. The regular sampling of pleurocarpous mosses for foliar response will give a long-term indication of whether N is impacting on the site. Tissue N concentrations in *Calluna vulgaris* or other dwarf shrubs may be informative for long-term integrity. However, care should be taken to sample for same age stands as the % N concentration in *Calluna vulgaris* varies with the age of the stand. Younger plants (0-6 years) have a higher % N than older plants.

Soluble ammonium content of foliage. (Note: A newly identified parameter, which appears to show a large response to enhanced N). As good a method as total tissue N concentration.

9: If trees present can do macrolichen frequency/composition.

10: Atmospheric N monitoring: Physical measurements are necessary to determine actual N inputs to the site so that management practices can be controlled and disturbances, which might contribute along with enhanced N deposition to changes in vegetation composition, are prevented.

The important measurements are wet deposition N (NH_4^+ and NO_3^- concentrations in precipitation. Measurements of NH_3 could be important if in an agricultural area.

14.2.4. Scenario 4. Assessing the impacts on a lowland site, which from the national maps shows an exceedance of the nitrogen critical load but where there is no strong local point source

The objective of this scenario is to assess if biomonitoring methods can be used to help in the judgement of whether nitrogen is affecting site condition presently or as an indicator of this in the long term. As 23 out of the 32 sites in the UK extensive study exceeded their habitat critical load this particular scenario has important implications for possibly a large number of SAC's throughout the UK.

Table 14.4. Preliminary decision tree for selection of nitrogen bioindicator methods for lowland SAC sites

1: To assess impacts of long-range N inputs at an lowland SAC site

a. **Assess the impacts** of enhanced N concentrations or deposition? *Go to 2.*

b. **Assess the magnitude** of atmospheric N concentrations or deposition? *Go to 7*

2: Obvious local source present: There is unlikely to be any local point sources at this type of site. The source will probably be long-range diffuse wet dominated N deposition. However, in agricultural areas the dry N deposition (as NH_3-N) will be as important to the overall total N deposition as wet N inputs from precipitation. If livestock are present, check stocking rates for local variation in N deposition. Determine N input for habitat from sources such as APIS.

Set up a number of replicate quadrats around the site for long term integrity/ species composition change monitoring.

3: Make *a species list* *of higher plants and bryophyte species* in the ground flora.

A species list of the site is important for long-term assessment of integrity changes but the use of the Ellenberg N Index is unlikely to give a strong indication of N impact at these diffuse N sites. If Ellenberg Index is applicable then determine the *mean Ellenberg N Index of higher plants and bryophyte species* for at least 5 quadrats at the site. (Note: Interpretation of the method may be complicated by local differences of habitat management and soil types, and is therefore best suited to application at sites with well defined site history.) This survey method would be beneficial as part of a temporal assessment, or wider spatial survey and not as a one off assessment. The use of the simplified acidophyte/nitrophyte method could also be a useful initial assessment.

4: Site wooded or trees present? *Go to 5*

Site treeless. *Go to 6.*

5: Simplified macrolichen survey of epiphytic species on twigs and trunks using revised indicator species for UK conducted by trained non-experts. *Method: Survey of key lichen species and calculation of acidophyte/nitrophyte scores.* (Notes: The method gives a broad indication of NH_3 exposure. Where possible sampling is done on acid-barked tree species on trunks and twigs in order to compare present conditions for lichen establishment on new substrata with established lichen communities on older substrata. See also Bark pH.

6: Standardised response to nitrogen deposition. *Method: Standardised grass plants (Lolium or Deschampsia).* (Note: the method is good for visual demonstration and directly records additional nitrogen absorption by the plants). For long-term application, a series of plants must be exposed over time. As the N inputs will be low, the use of standardised grass plants will require long-term exposure (6-12 months) and modified protocols to assess N impacts.

7: Trees absent *Go to 8*

Trees present *Go to 9*

Atmospheric monitoring *Go to 10*

8: Foliar responses to different forms of additional nitrogen. (Note that the dose response relationships probably differ for different N forms).

Total tissue N of foliage. (Note: suited to pleurocarpous mosses and certain well studied higher plants, e.g. *Calluna vulgaris*). The use of mosses and *C. vulgaris* are well-studied pollution biomonitoring parameters. Tissue N in moss species could be more related to concentration in the precipitation than N deposition. Due to possible seasonal variations in foliar N concentration and the importance of the hydration status of pleurocarpous mosses sampling should be carried out in late spring or early autumn. The regular sampling of pleurocarpous mosses for foliar response will give a long-term indication if N is impacting on the site. Tissue N concentrations in *Calluna vulgaris* or other dwarf shrubs may informative for long-term integrity. However, care should be taken to sample for same age stands as the % N concentration in *Calluna vulgaris* varies with the age of the stand. Younger plants (0-6 years) have a higher % N than older plants.

Soluble ammonium content of foliage. (Note: A newly identified parameter, which appears to show a large response to enhanced N). As good a method as total tissue N concentration.

9: Atmospheric N monitoring: Physical measurements are necessary to determine actual N inputs to the site so that management practices can be controlled and disturbances, which might contribute along with enhanced N deposition to changes in vegetation composition, are prevented. Measurements of NO_x concentrations could be important at this type of scenario when localised sources of NO_x are identified. The monitoring of dry gaseous NH_3 and also wet N deposition (NH_4^+ and NO_3^- concentrations in precipitation) are also required.

14.3. Conclusions

- The application of biomonitoring techniques at sites, which fit Scenario 1 (poultry farm on lowland SAC) have been shown in Section 8 to be robust and give an indirect indication of condition, but a direct signal of N impact on the SAC. They also give an'early warning' signal indicating potential changes and an effect on site integrity.

- A number of different biomonitoring methods could be applied in Scenario 1 depending on the habitat and the designated interest features.

- Biomonitoring methods could be applied to Scenario 2. The method used would depend on the habitat, interest feature and the concentration and type of atmospheric pollutant.

- The availability of a transect with uniform habitat is important for Scenarios 1 & 2 if the epiphytic or ground flora are to be used. Standardised plants give an indication of N impacting on the site but do not indicate the effect on the existing vegetation/interest feature. However, if the habitat is variable and the same indicator species cannot be found along the transect, then standardised plant could be used.

- Scenario 3 is the most difficult situation to apply biomonitoring methods. The diffuse wet N deposition sources together with possible climatic change and management practices make it very difficult to determine if N is impacting. The advantage of using foliar N concentration biomonitors would be to give a long- term record of N concentrations, which could give a reasonably robust indirect 'early warning' of potential impacts of N. As pleurocarpous mosses are prevalent in uplands habitats their use would give added value to CSM.

- As with scenario 3, the upland SAC's, the undefined diffuse source attribution of Scenario 4 makes it more complex to assess current and long-term changes due to N impacts. Other factors such as management practices, climatic variables could also be impacting on the SAC.

- The main difference between Scenario 3 and 4 is the potential level of N deposition. In Scenario 3, N was probably not impacting on the condition of the site, whereas, in Scenario 4 it is recognised as doing so (according to the scenario definition). However, in upland sites occult precipitation could increase NO_3^- and NH_4^+ concentrations by a factor greater than 2 and lead to critical loads exceedance. The use of biomonitors in Scenario 4 would give an indication of the current effect on the habitat and could also be used to determine changes in integrity if applied as part of a regular monitoring programme**.**

15. Assessment of nitrogen impacts on condition and integrity of four case study sites

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15.1. Introduction

The application of simplified biomonitoring methods in the intensive and UK-wide extensive studies (Sections 4-13) provided the opportunity to test these methods under field conditions in a wide range of habitats and N deposition levels distributed throughout the UK. Assessments of the individual methods used at the intensive and extensive UK sites are summarised in Sections 8 and 13. These sections determined the robustness and applicability of the selected biomonitoring methods for potential application by the conservation and environment agencies as additional monitoring tools for assessing N impacts site condition and integrity of designated statutory sites.

The objective of this section is to apply/link the results from the simplified biomonitoring methods and the atmospheric N and S inputs, to the condition and integrity of four case study sites in relation to the attributes for the interest feature of the specific habitat.

The four case study sites to be considered are Piddles Wood from the intensive study and Ariundle, Caldanagh Bog and Llanymynech and Llynclys Hills SSSI, three of the sites in the UK extensive study.

15.2. Background

15.2.1. Condition Assessment

The conservation agencies are responsible for the identification and protection of sites designated under national and European legislation (e.g. SSSIs, ASSIs, SACs, SPAs and Ramsar sites). Each site is designated on the basis of special interest feature/features, which could be a specific species, an assemblage of species, habitats, or earth science features. Once established as a designated site, one of the key roles of the conservation agencies is the continued protection of these designated sites. The condition of sites is assessed by the conservation agencies using common standards monitoring (CSM), whereby key attributes are identified and broad targets set for each feature at each designated site. The sites are monitored, on a six-year cycle, to assess the condition of the specific attributes and the feature as a whole.

Common standards monitoring is designed to be a ‘simple, quick, judgement-based assessment of the feature condition’ (www.jncc.gov.uk). It is important to note that the CSM assessment is for the feature of the designated site and not the site itself. CSM provides a number of categories for recording of the condition of interest feature.

- Favourable - maintained
- Favourable – recovered
- Unfavourable – recovering.

- Unfavourable – no change
- Unfavourable – declining
- Partially destroyed
- Destroyed

The CSM assessments from different sites are used to enable judgement to be made about whether the management of individual sites is appropriate and whether current legal, administrative and policy measures are effective.

CSM is not designed to detect and attribute the impacts of N deposition (or other air pollutants) on the features associated with the designated sites. One of the key aims of this current project was to determine if simplified N biomonitoring methods could be applied (in addition to CSM, environmental modelling or critical load assessment) at specific designated sites to determine if the feature/s are being impacted upon by increased N deposition.

The key requirements for the use of N biomonitoring methods would be a) simple to apply, and effective b) give added value (compared to critical load assessment) and c) be an integral part of the overall assessment of the condition and integrity of a designated site.

CSM defines a series of attributes and associated targets for each interest feature on site. An attribute is defined as ‘a characteristic of a habitat, biotope, community or population of a species which most economically provides an indication of the condition of the interest feature to which it applies” (www.jncc.gov.uk).

Targeted biomonitoring methods will provide an indication of the level of exposure and/or effect of N at a designated site. However, to be interpreted in the context of the existing monitoring framework (i.e. CSM) the identified effect needs to be related to the attributes of the interest feature for that designated site. Ideally, this would be a direct relationship (i.e. a measured impact on one or more of the attributes of the interest feature). However, in many cases it will be indirect and based on a judgement or association between the biomonitoring method and an ultimate response/effect on the attributes of the interest feature.

15.3. Site Integrity

Under the Habitat Regulations (1991), a competent authority (for example SEPA) must undertake an appropriate assessment for a plan or project (for example a PPC permit), which has the potential to impact on a European site, in order to ascertain whether there will be an adverse effect on the integrity of the designated site.

The current approach for assessing the potential impacts associated with emissions (either reactive N gases or other air pollutants) in relation to the tests of the Habitats Regulations is based on a risk assessment using ‘site-relevant’ critical loads. Therefore, a further aim of this project has been to consider the application of biomonitoring methods for assessing impacts on site integrity in relation to Habitat assessment and permitting conditions of authorised installations.

The definition of integrity is taken from DETR’s Planning Policy Guidance 9 where integrity at the site level is defined as “The coherence of its ecological structure and function, across its whole area, that enables it to sustain the habitat, complex of habitats and/or levels of populations of the species for which it was classified”.

15.4. Cause and effect biomonitoring conceptual model

If a designated site is suspected of being impacted upon by gaseous N concentrations or N deposition it is important to identify the N source for that specific site. Following source attribution the pollution-impact interactions with the designated feature or other potential indicators can be used to assess condition and integrity. Figure 15.1 outlines the source to response pathway for N bioindicators. This is explained in more detail in Section 16. This figure highlights that the strength of the link to source attribution and site condition/integrity increases once a physiological response has been identified. The diagram will be used as a framework in the condition/integrity assessment of the four sites using N bioindicator methods.

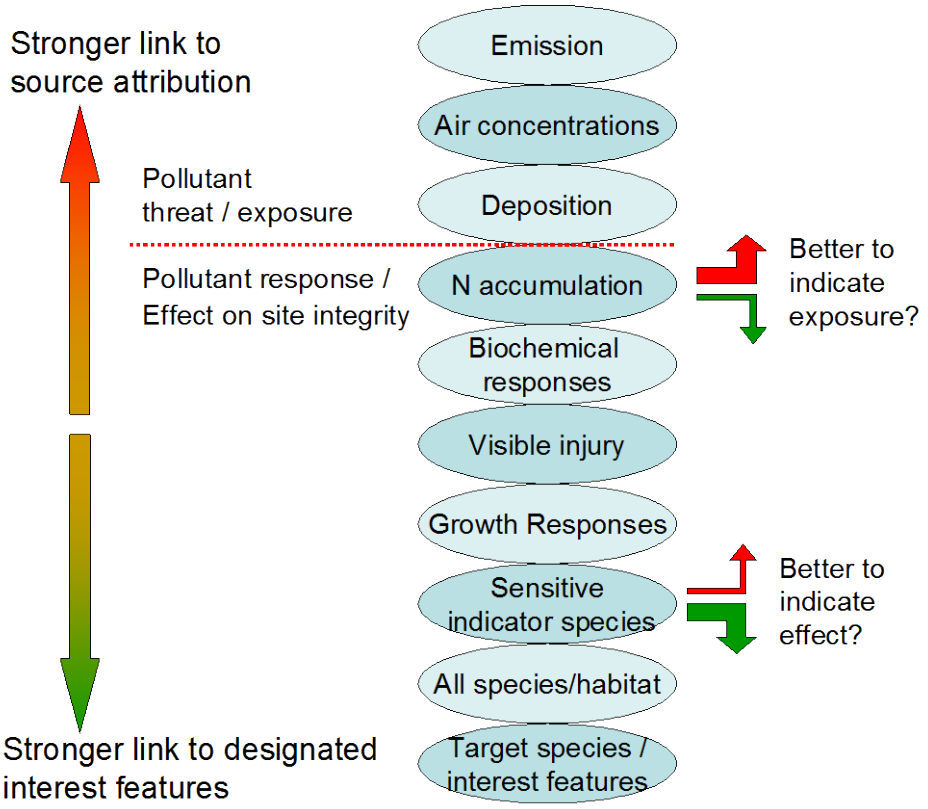

Figure 15.1. Cause/effect biomonitoring chain.

15.5. Piddles Wood SSSI

15.5.1 Nitrogen Source attribution

The major source of N deposition at Piddles Wood is gaseous NH_3 from the agricultural point source (poultry unit) situated in the SSW of the woodland. However, background deposition is also relatively high (26.2 kg N ha^{-1} y^{-1}) and exceeds the critical load for this habitat. The NH_3 concentrations and N deposition for this site are detailed in Section 3: Piddles Wood. There was a strong gradient in NH_3 concentration and total N deposition with distance from this point source (N deposition ranging from 1061 kg N ha^{-1} y^{-1} at 5 m from the poultry house to 26 kg N ha^{-1} y^{-1} at a distance of 250 m). It is recognized that there is a very high uncertainty in the largest deposition values at this site, for example due to the possibility of cuticular saturation effects, although this only becomes a major effect at deposition loads substantially larger than the critical load. For the

purposes of this assessment, the effects on condition and integrity are considered for the length of the transect only, and not the whole site.

15.5.2. Biochemical, physiological and ecological responses

Biochemical, physiological and ecological responses to elevated N in the form of NH_3 emissions from the adjacent point source were shown on the site by the use of a suite of biomonitoring methods including standardised plant growth and development, tissue N analysis (total N content and soluble NH_4-N concentration), pleurocarpous mosses, Ellenberg Index and lichen diversity.

15.5.3. Interest feature

The feature of interest for woodland is seldom a single vegetation type, as is the case for Piddles Wood, where the main habitat is a lowland broadleaved mixed and yew woodland. The site was notified as an SSSI largely because of its substantial oak woodland with coppiced hazel understorey. Thus the feature of interest could be said to be the woodland structure and species composition of woodland, shrub and ground layers. A full site description is given in Section 3.2.2.

15.5.4, Site Condition: Attributes

The attributes for woodland are defined and described in JNCC's commons standard monitoring guidance (www.jncc.gov.uk/page-2203/common standards monitoring guidance). For most woodland habitats, the conservation objectives define five broad attributes, which are **extent; structure and natural processes; regeneration potential; tree and shrub composition** and **indicators of local distinctiveness**. These are described in more detail below. The results of the biomonitoring methods study are then evaluated in relation to the above attributes.

Extent
This feature includes the distribution of the woodland feature across the site and is concerned with assessing the gross changes in the overall habitat extent. At Piddles Wood, the tree composition varied across the site with the site being divided into 4 management units. The tree species composition was relatively consistent along the study transect, except at 100 m from the farm NH_3 source, where more mature oak were found.

The Ellenberg index would give an indication of change in tree species composition, which could be directly related to N deposition. However, Ellenberg Index is probably more applicable for use in the **composition** attribute (see below). Similarly, the lichen species diversity method would indicate changes in lichen species composition but it does not indicate change in extent of the interest feature at Piddles Wood. The species composition measures and the foliar N methods are not applicable to the extent attribute of this interest feature. On the whole there is little relationship between the biomonitoring methods and this attribute.

Structure and natural processes
This attribute includes the extent to which the structure should be determined by natural processes rather than defined by management; the level of dead trees and the balance between canopy and shrub layers. Ellenberg Index could have an important role to play in this attribute. Ellenberg Index would highlight changes in shrub composition changes, which would then potentially change the canopy shrub balance. Accumulation and physiological indicators are also relevant for this attribute, since they demonstrate an alteration of natural processes.

Regeneration potential

This attribute includes the level of saplings and young trees expected to be seen and the extent of coppicing. This is an important attribute at Piddles Wood, as much of the unit where the poultry unit is located is regenerated coppiced woodland. Changes in shrub and ground flora species composition due to the increased N deposition from the poultry farm could lead to a reduction in regeneration of interest feature species. Potential alteration of the soil pH may influence seed germination of interest feature species. An increase in ground flora and shrub species may also influence sapling regeneration through increased shading and competition.

The foliar N concentration of the moss species would give an early indication of any potential N impact on the site. The increased total N content in *E. praelongum* and *E. striatum* within the woodland indicates increased N deposition to the woodland flora. This highlights the potential effect on target species including regeneration potential.

Composition

In the context of the interest feature for woodlands, CSM only refers to the species composition trees and shrubs and not the lower plant species, such as the lichen and pleurocarpous mosses. This attribute is targeted at the maintenance of particular species, especially native species. For example, while the understorey or shrub layer is dominated by hazel in the study area, further from the farm unit both Maple and Wild Service Tree are also present. The persistence and health of the latter species, which is relatively uncommon, could be monitored within the aegus of Ellenberg and N content of mosses. The results of the Ellenberg Index have shown that changes are occurring in the interest feature at Piddles Wood. One of the objectives of this attribute is to alert the agency of any rapid decline in species due to new diseases or insect infestations.

In this context, increased N inputs at Piddles Wood could be seen as a potential factor, which would alter the composition of the interest feature. The large concentrations of both total N content and soluble NH_4-N concentration in pleurocarpous moss species, which could be attributed directly to the presence of the poultry unit at Piddles wood, would also indicate that the woodland is being impacted by N. Previous studies (Pitcairn *et al*. 1998) have shown that total tissue N is increased in mature tree foliage in the vicinity of intensive livestock farms. Large trees are buffered from the effects of small changes, but long-term increases could lead to increased susceptibility to pest and pathogen attack, thus resulting in direct implications for site integrity.

Indicators of local distinctiveness

This attribute includes the ground flora composition, but not more than four species of importance, which have contributed to the selection as a SSSI/ASSI. In general, this could include a particular species or a transition zone to another habitat. For most woodland features the ground flora target will correspond to the relevant NVC type for that specific woodland habitat type. It would be considered unfavourable if there were changes in species composition, which were not consistent with the recognised NVC classification.

Piddles Wood is classified as a NVC class W8 *Fraxinus excelsior*, *Acer campestre*, *Mecurialis perennis.* Comparisons with Nature Conservancy Council surveys carried out at Piddles Wood (1985 and 1991), prior to the installation of the poultry unit, indicate a change in ground flora species composition. *Mercurialis perennis* (characteristic of the NVC classification) *Luzula sylvatica and Carex sylvatica* have declined close to the poultry unit and have been replaced by *Lamiastrum galeobdolon and Glechoma hederacea,* which are more N loving species However, changes in light and competition levels may also have contributed to the changes.

The further development and application of an acidophyte/nitrophyte index, where acidophytes are largely positive indicators for site integrity whilst nitrophytes are considered negative indicators, may be of considerable benefit to agency staff in assessing site condition.

The use of the foliar N content and soluble NH_4-N concentration methods using the pleurocarpous mosses also indicate that N emissions were is impacting directly on the site, however, the evidence for a localised effect close to the poultry unit (i.e. within a radius of up to 200-250 m around the unit). By the nature of using such a transect, with the furthest site used as a reference, it is not possible to show from the transect whether or not the source has impacts on the furthest site.

15.5.5. Summary–Condition attributes

- **Ellenberg Index** is most closely related to attributes of composition and indicators of local distinctiveness, less so the other attributes
- **Lichens** – are not really linked directly to the designated attributes for Piddles Wood but by inference they are a potential indicator of long-term changes in environmental conditions (see Section 15.5.6 describing integrity), which also relate to changes in natural processes
- **Pleurocarpous mosses** are similar to the lichen in that they are not directly related to the interest feature attributes but may be a useful tool as an early warning indicator (see section below) also indicating changes in natural processes.
- **Standardised grass biomonitors** –are not linked to the site attributes but conversely provide a graphic indication of the current level of N impact on the SSSI as a whole.

15.5.6. Integrity of site

English Nature's condition assessment of the site (January 2004**:** www.english-nature.org.uk/special sssi/site documents.cfm) classifies three out of the four units of the site as favourable. The woodland block that includes the woodland adjacent to the poultry house, is judged to be unfavourable due to the composition target failing because of the extent of conifer plantation in this area. The impact of N was not considered in the context of the CSM assessment.

The biomonitoring methods, particularly Ellenberg, can be related to the attributes and therefore some judgement is possible about impacts on condition (see above). However, biomonitoring methods could play an important role in identifying/alerting the agencies to potential long term change in attributes of the interest feature at Piddles Wood. In this context they are an early warning of future impacts on the attributes.

The bioindicator methods used in parallel at Piddles Wood were concentrated in the area adjacent to the poultry unit and along a transect running in a NE direction away from the poultry unit. All the methods used indicate that N deposition is impacting on the woodland in 250 m radius from the poultry unit (based on the transect sampling approach used, it cannot be stated whether or not there are significant impacts beyond this distance). The high N levels in the pleurocarpous mosses close to the unit and the species present indicate that species composition changes have probably already occurred.

The lack of pleurocarpous moss species diversity close to the unit would indicate that only those species with a high N tolerance are now able to exist. Long-term N accumulation could lead to changes in the moss species composition. Work at the Whim moss manipulation study has shown accumulated NH_3-N deposition will reduce species composition at increasing distances away from the point source with time. The high N levels in the pleurocarpous mosses close to the unit also imply larger than normal concentrations of N in the soil, which will affect, not only species composition of the ground flora, but also the health and reproduction of the species of the shrub and tree layer.

The long-term integrity of the interest feature is thus expected to be affected by the N impacts. The results of the Ellenberg Index study indicate that N is currently impacting on the ground vegetation close to the poultry unit. Long-term accumulation of N deposition at this site would lead to an increase in nitrophytic species at greater distances from the poultry house, which is an indirect impact on site integrity but could have implications for the interest in the long term, including species composition changes.

The lichen survey has shown the importance of epiphytic lichens on twigs in assessing the impact of ammonia on twig lichen communities at distances of c. 250 m where the measured ammonia is only c.1.5 µg m^{-3}. The use of twig and trunk monitoring on acid barked tree species allows the assessment of the status of indicators of ecological continuity on the trunks and the long term potential for change from the lichen community on the twigs.

15.6. Caldanagh Bog ASSI

15.6.1. Characterisation of N inputs

The N inputs at this site are dominated by NH_3 dry deposition from agricultural sources (mean NH_3 concentration 2.58 µg m^{-3}) leading to an estimated NH_3-N dry deposition input of 13.9 kg N ha^{-1} $year^{-1}$. Overall, the mean total N deposition for this site including wet deposition inputs is 22.1 kg N ha^{-1} y^{-1}, which exceeds the critical load for a lowland raised bog (5-10 kg N ha^{-1} y^{-1}). The expected changes would be changes in species composition and N saturation of *Sphagnum* species.

15.6.2. Interest feature

The interest feature at Caldanagh Bog (Northern Ireland) is the lowland raised bog. The following citation was extracted from www.ehs.gov.uk/natural/designated/site view.asp.site noASSI123. 'Caldanagh bog is a compact lowland raised bog within the River Main series displaying a classic domed profile with minimal turf cutting around the periphery. An area of intact lagg along the north-eastern edge of the bog represents one of the most important features of the site. The intact surface supports a moderately well developed hummock/hollow complex and the surface of the bog is exceptionally wet supporting a dense and diverse cover of *Sphagnum* mosses.

Of particular note, the nationally rare *Sphagnum pulchrum* is abundant in the hollows and the notable hummock-forming mosses *S. imbricatum* (*S. austinii* and *S. affine* are components of the *S. imbricatum* complex) and *S. fuscum* both occur. Some of the peripheral peat has been cut for turf in the past with vegetation communities ranging from deep artificial pools to *Molinia caerulea* dominated grassland.

The overall diversity of Caldanagh Bog is enhanced by a small esker ridge to the southwest, where the vegetation is dominated by a heath and grassland mosaic. The notable Burnet-saxifrage *Pimpinella saxifraga* grows on this esker ridge'.

In addition NVC communities are used as indicators of condition.

15.6.3. Attributes and targets

For all lowland wetlands the mandatory attributes are;

Habitat extent
The extent target should be no reduction in the area of the bog. Aerial photography surveys are the efficient means of surveying habitat extent. The biomonitoring methods would not give any indication of change in extent.

Habitat composition
This attribute is concerned with component habitats, whereby a site could have more than one component wetland type present. This is possibly the case with Caldanagh bog with the lagg fen feature. The target aim of this attribute is to sustain the variety and extent of the components. While aerial photography surveys will show changes in the extent of the habitats, the simplified acidophyte/nitrophyte Ellenberg N Index approach developed in this study could be used in this situation to determine changes in the balance of species particularly *Sphagnum* species and potential impacts from N. Such an adapted Ellenberg Index would concentrate on the presence/absence of key acidophyte and nitrophyte species. Foliar N concentrations could also be assessed on the *Sphagnum* species, using bioindicator methods, to provide an early indication of N impact, which may lead to a decline in the diversity of the *Sphagnum* species.

Habitat structure and natural processes
Deterioration in the raised bog structure can be caused by a variety of localised activities, such as peat cutting, drainage and over-grazing. The interest feature citation would indicate minimal turf cutting around the periphery of the raised bog. Visual estimates of exposed substrate could be included in an Ellenberg species determination. Again mosses and lichens are indicative of changed natural processes.

Vegetation composition
Recommended methods of assessment for positive and negative indicators include visual assessment of cover using a structured walk or transects and recording quadrats. Such an approach and the resulting species lists and cover estimates could be used for the purpose of determining a N Index for vascular plants, bryophytes and ground lichens. For more rapid assessment, a modified Ellenberg acidophyte/nitrophyte Index may be determined. If assessment was carried out along a transect across the bog, changes in the N index along the transect might indicate changes resulting from local sources of N.

A transect may be used for other bioindicator tests such as tissue N content and soluble NH_4-N concentration of pleurocarpous mosses and also transplantation tests. Although, considered a specialist technique, transplantation of *Sphagnum* moss from a known clean site to a test site and the subsequent monitoring of N accumulation can be readily carried out with the use of 'netlon' cylinders. Transplantation of Sphagnum has proved successful in the glasshouse (CEH) and field (Woodin, Press & Lee 1985, Mitchell *et al.* 2004, Leith *et al*. 2003)

Monitoring of species diversity changes in N sensitive bryophyte species could also be beneficial as an addition tool to CSM if a potential eutrophication problem was identified.

The results from the biomonitoring would give an early warning of potential N impacts to the habitat.

Indicators of local distinctiveness
This attribute is defined as the 'features of the heathland, that make it special but which are not covered by the attributes already described' (JNCC, CSM for Lowland Heathland 2004). A number of the N biomonitoring methods could be used under this attribute to add to the CSM assessments if the 'special' features were applicable to the N biomonitoring methods.

15.6.4. Integrity

The long-term integrity of the site is principally dependent on the continued ombrotrophic status of the raised bog. The characteristic N sensitive bryophytes and lichens species of the habitat are dependent on the maintenance of the hydrology state and the eutrophication status.

The use of regular foliar N monitoring could act as an early indicator of change in condition of the feature species. Long-term eutrophication will lead to species composition changes and loss of integrity. The current N deposition inputs to this raised bog indicate that N could be impacting on the condition of the bog. The levels of foliar total N measured in *R. squarrosus, Scleropodium purum* and *Thuidium tamariscinum* were 1.56% N, 1.14% N and 1.56% N respectively. These concentrations, which are higher than expected for a raised bog habitat, would suggest N is currently impacting on the bog and surrounding area. If these species are being affected by increased N, the potential impact for the feature *Sphagnum* moss species, which are more N sensitive than the pleurocarpous moss species, is much greater.

Additionally, the trunk and twig lichen assessments both indicated lichen flora dominated by nitrophytes. Although these measurements do not link directly to the designated features of Caldanagh bog, they provide useful biological additional evidence, which supports the interpretation that the integrity of this site is under significant threat from enhanced NH_3 and N deposition.

15.7. Ariundle SSSI

15.7.1. Characterisation of N inputs

Ariundle is situated in the west central Highlands of Scotland, an area dominated by high precipitation and diffuse wet N deposition. This site has the lowest ambient NH_3 concentrations (0.04 $\mu g\ m^{-3}$) of the 32 Extensive UK sites and one of the lowest total N deposition values (11.3 kg N $ha^{-1}\ y^{-1}$). Although, the NH_4^+ and NO_3^- concentrations in precipitation are low, these combined with the high annual precipitation inputs result in the annual mean N deposition of 11.3 kg N $ha^{-1}\ y^{-1}$.

15.7.2. Interest feature

The description below is based on the SNH information for the Sunart Site of SSSI, Lochaber, Highland. Using the JNCC guidance the designated interest feature will be the western acidic oak woodland and mixed woodland on alkaline soils associated with rocky slopes. This habitat type has rich lichen, moss and liverwort communities that are included in the site outline of non-vascular plant interest.

Ariundle is mature deciduous woodland dominated by oak, birch and ash with an understorey of rowan, hazel and holly. The moine rocks and acidic soils support a rich flora dominated by *C. vulgaris, Vaccinium myrtillus, Deschampsia flexuosa* and *Pteridium aquilinum.* Base rich flushes support *Parnassia palustris* and butterfly orchids *Platanthera* spp.. The western Atlantic oak wood is an internationally important habitat with a rich diversity of ferns, mosses, lichens and liverworts including many 'Atlantic' species in all groups including lichens *Biatora vernalis* (NR*) Degelia atlantica, Hypotrachyna taylorensis, Menegazzia terebrat* and *Pseudocyphellaria norvegica* (NS*),* ferns; *Dryopteris aemula,* bryophytes *Dicranum scottianum* and *Sematophyllum micans.*

15.7.3. Attributes

The attributes used for Ariundle are based on the JNCC Common Standards monitoring Guidance for Woodland Habitats (www.jncc.gov.uk).

Extent
The Ariundle SSSI is part of the Sunart SSSI, which covers an area of 5500 hectares. Sunart SSSI is one of the most extensive areas of natural ancient semi-natural woodland in the UK.

There is no indication as to the extent status of this SSSI in the SNH documentation on this site. It is suspected that any change in extent could be driven by modifications in climatic factors, management practices as well as increased N inputs. In a diffuse wet N deposition dominated site such as this, the link to source attribution is very weak (Figure 15.1). With the presence of a rich diversity of non-vascular plants, biomonitoring methods could be utilised to give early warning of changes in extent. The exposure of *D. flexuosa* standardised plants would indicate if N deposition is impacting on the understorey vegetation. The advantage of using *D. flexuosa* is that this species occurs naturally in this habitat so any change in foliar N status could be directly related to N impacts on the SSSI. Changes in N status of mosses could also give early indication of potential changes.

Structure and natural processes
The Atlantic oak woodland habitat is dominated by the high rainfall and the mild climate of the west coast of the UK. Any major change in structure and natural processes is likely to result from modifications in these climatic factors. Although, it is difficult to directly assign effects of diffuse N deposition, it is important not to exclude the influence of N deposition on these habitats (Mitchell *et al*, 2004). The lichen and moss N concentrations could be used directly as part of the long-term monitoring of structure change in this habitat, since these represent key interest features to the structure of the site. In addition, any changes to the bryophyte and lichen communities are indicative of alteration in natural processes.

Regeneration potential
This attribute applies to the regeneration of the tree species and not ground vegetation. The regeneration oak woodland saplings at Ariundle are probably more dependent on the grazing pressures from the deer population and climatic conditions than from N impacts. However, changes in ground flora (moss and lichen species diversity) through increased N deposition could impact on regeneration potential by altering the microclimate on the woodland floor for seed germination/sapling growth. Biomonitoring could contribute to an early warning of potential change.

Composition
This attribution has importance for Ariundle as it considers change in natural species. The interest feature at Ariundle is the western acidic oak woodland and mixed woodland. As with regeneration potential other factors will have a greater influence on the composition of the feature than the N impacts.

Indicators of local distinctiveness
This attribute includes the ground flora, of which non-vascular plants are an important component, making it a species-rich site of international importance and a refuge for many species dependent on low nutrient budgets and high rainfall and relative humidity. Potential changes in species composition are an important additional factor in the assessment of this type of habitat.

The use of biomonitoring methods such as the foliar N concentration, modified Ellenberg acidophyte/nitrophyte Index and lichen indicators could give added value to the CSM of this habitat type. In particular, the detection of any loss of acidophyte lichen species would indicate a threat to all low nutrient species in this habitat prior to the arrival of nitrophytes.

The importance of the epiphytic twig flora should be emphasised, as this will provide a mechanism for detecting ongoing changes in atmospheric conditions in the vicinity of species-rich cryptogamic communities, while trunk communities provide information on ecological continuity. The routine application of the foliar N methods would give an early indication that N was impacting on the moss species and would also give an indication of the potential long-term threat to the interest feature from N deposition. Care would have to be taken in interpretation of the foliar N data because of climatic and seasonal variability in N concentration. Measurements would have to be carried out at the same time of year and when the hydrological status of the moss was consistent with previous samples.

15.7.4. Integrity

In the case of Ariundle, the regular use of N biomonitoring methods would provide a general early indication of potential long-term site condition change, through increased N impact, but not specific condition changes to the interest features. Recent work has shown the effect of long distance transport of NH_4^+ compounds on acidophyte species such as *Bryoria fuscescens* > 1000 km from source (van Herk 2003) suggesting that biomonitoring of acidophytic lichens will act as an early warning system to detect ongoing environmental changes. Although, the ground vegetation and especially the lichens and mosses are initially more sensitive to N impacts than the trees species, increased accumulated N deposition over a long period could impact on the interest features. The total N content (% N) and soluble NH_4-N concentrations measured in pleurocarpous mosses at this site indicate a low N impact on this habitat at present (foliar N contents < 1% N and NH_4-N concentrations < 4 μg g^{-1} FW). The effects on long term eutrophication on the soils of this habitat could be important for long term integrity of this site and merit regular biomonitoring.

15.8. Llanymynech and Llynclys Hills SSSI

15.8.1. Characterisation of N inputs

The measurements of NH_3 concentration at this SSSI have shown a mean value of 1.8 μg m^{-3}, with modelled total N deposition of 20.5 kg N ha^{-1} y^{-1}. Approximately 45% of the N input is as dry NH_3-N deposition. This is a hill site adjacent to intensive agricultural plains which provide a significant regional source of atmospheric ammonia.

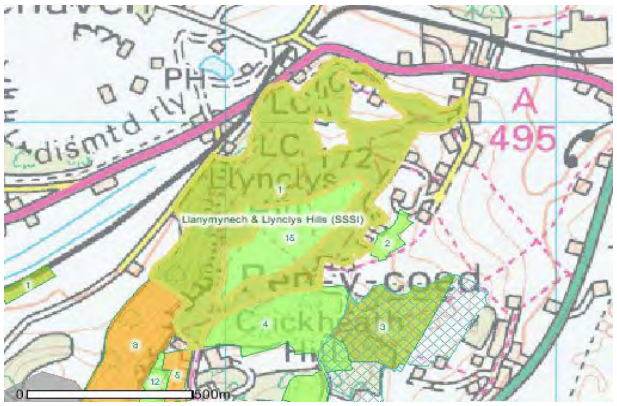

Figure 15.2. Map of Llanymynech and Llynclys Hills SSSI. (taken from www.en.gov.uk).

15.8.2. Interest feature

The Llanymynech and Llynclys Hills SSSI, in Shropshire is divided into 15 management units by English Nature, covering a total area of 106 ha (Figure 15.2).

It has been selected as one of the SSSI's to be assessed because it has a complex of interest features. They are: a) broadleaved, mixed and yew woodland upland, b) neutral grassland lowland c) calcareous grassland lowland.

The mosses and lichens were sampled in the broadleaved, mixed and yew woodland and the neutral grassland lowland.

Lowland calcareous grassland was selected as the interest feature for comment in this section because the site is particularly important for limestone plants. The grassland communities represented are extensive and varied and include many uncommon vascular species including orchids, lichens and grasses. As many as 10 NVC types are included within the Lowland calcareous grassland feature. From the limited material available, the exact NVC classifications for the areas of limestone grassland at this site are not known, but it is likely that more than one is represented.

Extent
The extent target should be no reduction in the areas of calcareous grassland. Aerial photography surveys are the efficient means of surveying habitat extent. While biomonitoring methods may not give any additional indication of change in extent, the rich diversity of vascular plants at this site could be measured by early warning of changes provided by biomonitoring methods. The exposure of *D. flexuosa* standardised plants or changes in the foliar N content of key bryophytes and lichens would indicate if N deposition is impacting on the understorey.

Sward composition-Grass: herb ratio
This attribute is usually carried out by some method of structured observation. A modified Ellenberg acidophyte/nitrophyte index at fixed points could contribute to this assessment:

Frequency of positive indicators
Monitoring of this attribute involves selecting 2-6 representative species and monitoring their frequency. For this site, species such as *Primula veris, Helianthemum nummularium, Scabiosa columbaria,* orchid species and *Cladonia* lichen species might be chosen. These species have low Ellenberg numbers and could all be considered as acidophytes.

Frequency of negative indicators
There are 5 groups of negative indicators: agricultural weeds, agriculturally favoured species, rank grasses, introduced species and native scrub and tree species. Species from the first 3 groups are likely to have above average Ellenberg N scores and could be considered nitrophytes. For example, *Brachypodium pinnatum* while scarce at this site is not uncommon at other Midland sites. This species is known to respond to increased N deposition at the expense of low growing species (Pitcairn *et al*. 1991) and hence may affect the species diversity of the sward. It is clearly important that such a species is regularly monitored. While it may be preferable to monitor each negative species, an acidophyte/nitrophyte index would provide a more rapid test, which could be applied more regularly.

Indicators of local distinctiveness
The maintenance of existing populations of rare/scarce species could be included in the application of a modified Ellenberg Acidophyte/nitrophyte index. Height, litter and bare ground parameters could be incorporated into an Ellenberg or modified Ellenberg cover survey.

15.8.3. Integrity

Ellenberg N Index has proved a useful indicator of temporal change in species diversity. Annual application of this test and/or a modified acidophyte/nitrophyte index would contribute considerably to assessment of site integrity. Changes in epiphytic lichen communities on calcareous substrata have still to be assessed in a national context in terms of their association with atmospheric nitrogen especially if bark pH is affected and in conditions where acidophytes are infrequent.

Within this section foliar N content of key bryophytes and lichens could provide a clue to the health of the positive indicators. The current total N content (1.1% N) and soluble NH_4-N concentrations (< 4 $\mu g\ g^{-1}$ FW) measured in pleurocarpous mosses indicate a low N impact at this site at present. However, the measured lichen species composition at this site indicate that the site is affected by nitrogen eutrophication, with AV-NV scores of -0.2 and -1.2 for trunks and twigs, respectively. These conflicting results may indicate a site, which is on the borderline of adverse effects on its integrity. Continued biomonitoring should provide a valuable indication of long-term integrity of the site.

15.9. Conclusions

- The conservation agencies are responsible for identification and protection of sites designated under national and European conservation legislation. Sites are designated for their specified interest features.

- The condition of sites is assessed by the agencies using common standards monitoring (CSM), which focuses on simple quick monitoring of key attributes, carried out on a 6 year cycle. CSM is not designed to detect and attribute the impacts of atmospheric nitrogen deposition or other air pollutants.

- Biomonitoring methods for nitrogen could be applied in addition to CSM at specific designated sites to support the assessment of whether interest features are being impacted by increased atmospheric nitrogen concentrations and deposition. For this purpose they should be a) simple and effective, b) give added value compared with modelled critical loads assessment and c) be an integral part of the assessment of condition and integrity of a designated site.

- Biomonitoring approaches can be considered in the context of the pathway from the source to ultimate effects on designated interest features. The concept of a “biomonitoring chain” highlights how a carefully designed program of biomonitoring can both make the link to source attribution and demonstrate impacts on relevant interest features.

- Five key attributes are relevant for the CSM, to which N bioindicators may be related:
    - Extent of the interest feature in a designated site
    - Structure of the interest feature and natural processes
    - Regeneration potential
    - Tree and shrub composition (or composition of other key interest features)
    - Indicators of local distinctiveness.

- Four examples sites are considered from the present study to address the relevance of nitrogen biomonitoring to site condition assessment. The sites chosen were Piddles Wood (Dorset), Caldanagh Bog (Northern Ireland), Llyncis Common (Shropshire) and Ariundle (Scottish Highlands).

- The examples demonstrate how different bioindicator methods make the link to site condition either directly (e.g. Ellenberg indicators for higher plants) or indirectly (e.g. moss chemistry as an indicator of altered natural processes). They also demonstrate the problem that in some cases relevant bioindicators are not directly relevant to the interest features: For example, moss and lichen assessment is directly relevant to the designated Atlantic flora at Ariundle, but not directly relevant to the designated woodland habitat at Piddles Wood. In these cases, such biomonitoring methods still have value (by increasing the robustness of the assessment), and can be taken as "early warning" of changes to the designated features.

16. Future challenges: the importance of benchmarking and intercalibration for integrated application of nitrogen indicators by conservation agencies

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16.1. Introduction

This report has described in detail the results of intensive biomonitoring for nitrogen at 4 test sites plus the application of the moss chemistry and lichen diversity methods at the UK scale. These have provided the basis to consider protocols for the application of nitrogen biomonitoring in example scenarios and to show how biomonitoring can be used to assess the consequences of elevated nitrogen input for the integrity of designated sites.

The analysis shows that, while some biomonitoring methods are directly relatable to designated features of sites, others are only indirectly relatable. For example, application of the Ellenberg scoring method for higher plant species composition is relevant where the CSM target includes sward or woodland understory composition as a designated feature. By contrast, the measurement of epiphytic lichen species composition is only directly relevant at the few sites where lichens are included as part of the site designation. A further difficulty is that the biomonitoring methods most relevant to condition assessment tend to provide the weakest link to the cause of any changes, since observed changes may also be driven or modified by several factors other than air pollution.

In the first instance, it can be considered that improved robustness in biomonitoring for nitrogen can be found by using *several methods in parallel*. If all the methods agree, this provides a more convincing assessment. Such a basic combination of methods, however, does not address the link between monitoring of condition and N deposition as the driver of change. Similarly, it does not answer the question of how the results of a biomonitoring method that is not directly related to site condition can still be used as a quantitative indicator of threat to site condition.

These issues provide the basis to address the future challenges of *benchmarking* and *intercalibration* of N bioindicators. In this section, the concept of the nitrogen "biomonitoring chain" is used as a starting point to develop the basis for benchmarking and intercalibrating bioindicators and to visualize approaches for combining the results of different bioindicator methods. A framework emerges that provides a skeleton for the integrated application of bioindicators, while the key uncertainties in the framework help identify priorities for future development.

16.2. The biomonitoring chain and its implications for linking source attribution and effects on site condition

While an element of robustness in biomonitoring can be found by using the results of several methods, the interpretability of results can be further improved by considering the pathway from air pollution emission to ultimate effect on site condition. Each of the bioindicator methods can be placed at different stages in this pathway, which naturally also includes physico/chemical indicator methods. Figure 16.1 envisages the pathway as a "biomonitoring chain", where measurements can be made at 10 different stages. The first three "links", represent the pollutant threat or exposure, while each of the others represent biological responses of the system and potential effects on site integrity. However, this simple two-way distinction is less helpful than the fact that logical connections apply between each of the stages along the chain.

The biomonitoring chain concept is highly relevant to the practical application of indicator methods. Although the chain is applied here to nitrogen, it is equally relevant for monitoring of other air pollutants and their impacts. Conversely, it must be recognized that not all stages in the chain apply in every context. For example, impacts of N deposition on species composition will often occur without obvious visible injury. In relation to ozone (O_3) impacts, visible injury may occur, but these cannot always be related directly to changes in plant growth. It is important not to get distracted by such obvious exceptions, but instead focus on the wider implications the biomonitoring chain.

The first implication is that, instead of robustness being provided by a random collection of several methods, it is advisable to *select monitoring methods widely distributed along the biomonitoring chain*. Methods toward the top of the chain are better suited to indicate exposure and make the link with source attribution, but provide a weak link to impacts on designated features. Conversely, methods toward the bottom of the chain are better suited to indicate effects on the designated features, but provide a very uncertain link to the cause of change. Hence by selecting several methods distributed along the chain, the logical cause-effect link is strengthened. Such a structured approach to biomonitoring might show at a certain site that:

a) relevant impacts on target species and site condition are occurring,

b) these impacts can be related to biochemical changes reflecting a perturbation of the natural processes at a site,

c) the biochemical changes can be related to exposure of a certain pollutant, and finally that,

d) the high level of a pollutant can be related to emissions from a certain polluting activity.

Of course, such a chain of measured indicators does not prove causality between emission and impact on site condition. Nevertheless, it provides a suitable framework within which causality can most effectively be assessed.

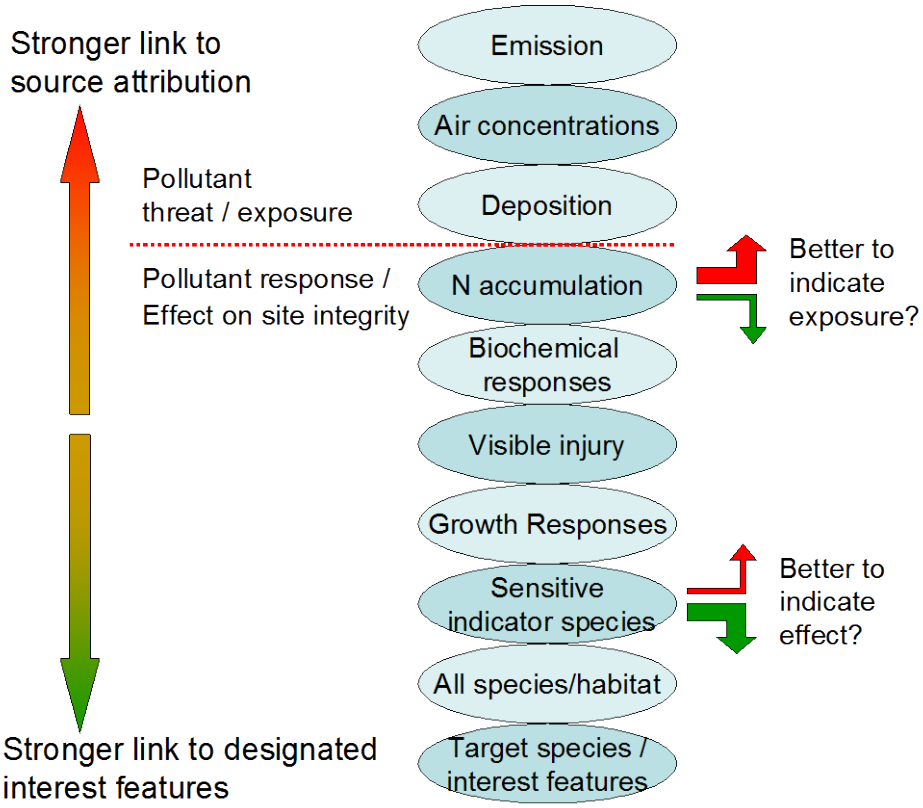

Figure 16.1. Overview of the "biomonitoring chain" showing how different indicator measurements may be ordered from pollutant source to ultimate pollutant impacts. Measurements closer to emission show a stronger link to source attribution, but weaker link to effects on designated interest features. Conversely, species-based measurements show a close link to the designated interest features, but a weak link to source attribution. A comprehensive robust program of biomonitoring should therefore combine measurements distributed along the biomonitoring chain. The dark shaded ellipses show measurements that are, in general, the most practicable.

The second practical point to note about the biomonitoring chain is that some links are harder to measure than others. Fortuitously, the easiest parameters to measure are well distributed along the chain. Thus it is difficult to monitor emissions from many types of air pollution source (e.g. road, farm, complex factory sources etc), but it is straightforward to measure atmospheric concentrations. Similarly, measurement of atmospheric deposition is a complex issue with many uncertainties, but estimates can be directly derived from or related to atmospheric pollutant concentrations.

Continuing down the chain, it may be noted that measurement of pollutant accumulation is in many cases much easier than determining the biochemical response. In the context of nitrogen, the measurements of total foliar N content and soluble NH_4^+ concentration have both been shown to be straight-forward (Sections 8 and 10), and these are easier to measure than responses such as the activity of the enzyme nitrate reductase. The biochemical responses also tend to provide a poorer measure of pollutant exposure than simple biochemical accumulation (Sutton *et al.* 2004a). This is because they are affected both by pollutant accumulation and other constraining factors, such as light, soil type and water availability.

Where relevant, visible injury is straight-forward to record, given appropriate training, however, it does not always represent the possible extent of impact and in addition many impacts to chronic exposure can induce change without visible injury occurring. Although injury is not always an issue at sites affected by chronic nitrogen deposition, injury can occur in the field, for example with high NH_3 concentrations leading to characteristic visible injury to mosses and lichens (I.D. Leith pers. comm.). By contrast, measurement of growth responses of in situ vegetation is very labour intensive and not suited to practical application in support of CSM. Assessment of growth

rate changes can be extremely useful, however due to the resources required such measurements are limited to situations where an extremely detailed assessment is justified (e.g. Mitchell *et al.* 2004). An exception is the measurement of growth responses and N inventories of standardised grass 'biomonitors' placed in the field (Section 5 and Appendix II), which can provide a rapid demonstration of impact particularly useful for regulators (i.e. environment agencies) and local stakeholders.

Recording the response of sensitive 'indicator species' can provide a very useful and cost-effective method for assessing the impacts of N air pollution on a given site. By definition, in using the most sensitive species, such responses do not necessarily imply an adverse effect on the designated features of a site. However, they do provide the clearest signal of actual species change. Examples include the use of epiphytic lichen species composition as an indicator of ammonia (Section 7 and 11), or the use of acidophyte and nitrophyte species in higher plant ground flora (Section 4).

Complete monitoring of all species in a designated site is, of course, labour intensive, and this is why CSM tends to focus on a more rapid assessment of target species and/or key interest features. The last link in the chain is thus closely related to CSM, although additional measurements of the target species/ interest features may be relevant in the context of assessing air pollution impacts. For this purpose, starting point may simply be to emphasize the known links between existing CSM monitoring and signals of air pollution impact.

16.3. The need for benchmarking and intercalibation of bioindicator methods

The biomonitoring chain thus provides a useful framework for linking source attribution and effects on site condition. By contrast, it has been noted that being able to make these linkages does not necessarily imply causality between emissions and effects on site condition. Similarly, there remains the question of how quantitatively to relate signals recorded in one part of the biomonitoring chain to signals in another part of the chain. This second problem is well understood in relation to the limitations of physical monitoring. For example, high NO_x concentrations or critical load exceedance do not automatically imply that deterioration of a certain site is currently detectable. However, the problem is not limited to this comparison. Rather, the problem applies to the relationships between all the stages in the biomonitoring chain. Hence, the question can be asked how a high value of foliar N quantitatively relates to both N deposition and to changes in site condition. Similarly, the lichens most sensitive to NH_3 may disappear from a site, but how does this relate to changes in woodland ground flora, the health of oak trees or some other designated feature?

These issues point to the need for benchmarking the results of each of the indicator methods and for improving the intercalibration between their results.

Benchmarking refers to comparing a given bioindicator result against a standard, in particular, defining the *critical values* for each of the indicators in different contexts. These critical values need to be known for each indicator, including how they vary by context. The benchmarks for air concentrations and deposition of N are well known (critical levels and critical loads), but much more effort is needed to develop the benchmarks for different bioindicator methods.

Intercalibration refers to the setting of the benchmarks so that the results of different indicators can be quantitatively compared. This has two parts:

a) *Setting the zero point*: the benchmarks for different indicator parameters should be set so that they reflect a *common standard of effect* on the habitat. This is to say that the point of exceedance of the critical values for the different indicators should occur at the same position on the dose-response function.

b) *Setting the range scale*: the scales for the indicators should be set so that a certain degree of exceedance is comparable between different methods. For example, if several of the bioindicator methods show 150% of the critical values at a site, each 150% should ideally relate to a common point in the dose-response function.

Benchmarking and intercalibration between indicators provide the essential *basis to relate the results of one indicator to another*. Through benchmarking and intercalibration to a common standard, the signals recorded by, for example, epiphytic lichens could be quantitatively related to foliar N concentrations of mosses, atmospheric N concentrations or deposition and vegetation change in woodland ground flora.

Establishing the benchmarks and intercalibration is equally a prerequisite to address the *causality between emission and effect* on site integrity and condition. It was noted above that applying indicator methods well-distributed along the biomonitoring chain does not necessarily imply a causal link (even if there is both a known loss of site condition and known pollutant source). However, if the same suite of indicators is applied, where these have previously been intercalibrated, then consistent exceedance along the biomonitoring chain provides a much stronger evidence base to assess causality.

16.4. Dealing with different units between indicators in benchmarking and intercalibration

One of the major challenges in setting benchmarks and intercalibrating between indicators is that each indicator is measured using different units. The units of atmospheric deposition (e.g. kg N ha^{-1} y^{-1}) differ to those for air concentrations (e.g. μg m^{-3}), while even the different N accumulation parameters use different scales, such as foliar N content of foliar NH_4^+ concentration as μg NH_4-N g^{-1} FW. The intercomparability appears even harder if the species-based assessments are considered, such as Ellenberg score or acidophyte-nitrophyte values.

Potentially, this could give rise to a multi-dimensional comparison between all the different parameters, which substantially complicates the intercalibration process. Ideally, therefore, the intercalibration should be based on as few unit scales as possible. The unit scales should be naturally quantitative and ideally be relatable to source activity and its mitigation. From this perspective, the natural choice for such a “common currency” is to, as far as possible, base the benchmarking and intercalibration on the units of atmospheric N deposition and air concentrations.

There are different advantages of each of these scales:

- *Atmospheric N deposition* has the advantage that it integrates all forms of N input. However, it has the disadvantage that it is complex and uncertain to estimate, while different N forms may vary in their effects.
- *Atmospheric reactive N concentrations* have the advantage that they are simple to measure accurately and can be related to specific dose-responses. However, they have the disadvantage that effects are often the consequence of more than one form of N including both dry and wet deposition.

Both scales are in general well suited because the scientific experiments that assess impacts are based on responses to deposition or air concentrations. This also facilitates inter-comparability between the practical application of biomonitoring methods and the critical loads/levels approach.

The use of deposition and air concentrations as “common currency” may superficially appear to blur the distinction between the critical loads/levels and the biomonitoring approaches. The two approaches do, however, remain fundamentally different:

- the critical loads/levels approach provides a risk assessment for expected impacts (which are not actually measured), even if it is applied using site-relevant estimates of concentrations/deposition and critical levels/loads.
- the bioindicator approach provides measurements of biological response that give evidence of actual change in site condition.

Having noted this, both bioindicator measurements and the use of site-based critical loads/levels provide complementary components in an integrated assessment using the indicator chain approach.

Some bioindictors respond most closely to N deposition, while others respond more closely to air concentrations of N gases. Additionally, the two scales are not consistently proportional, since the partitioning of wet versus dry deposition varies widely between sites. For this reason, while air concentrations and deposition may provide the units for intercalibration, it is helpful to envisage results in a unit less dimension, e.g. as percentage of the critical value. Hence hypothetical values for all indicators at 100% would imply a site exactly at the critical point. Where values for all the indicators are <100%, this would imply a site in favourable condition/ not under threat, while values consistently >100% would imply a site under threat and with demonstrable impacts on site integrity.

Although air concentrations and N deposition can provide common scales for many of the indicators, they are increasingly difficult to apply further down the biomonitoring chain, where the link between indicator and source is most distant. In these cases, such as Ellenberg score or CSM results, different units are needed, although the aim is to apply the same standard of effect as for the other indicators. In the intercalibration of such scales, setting the critical value for the benchmark is (conceptually at least) straightforward. For this purpose, the concept of the critical load as the deposition below which effects are not observed (according to current knowledge) provides a well understood standard. By contrast, it is a significant challenge to intercalibrate the range-scales to give comparable percentage exceedance values.

16.5. Relevance of spatial and temporal analysis for intercalibration of indicators

There are four main ways in which bioindicators may be used to assess impacts of N on conservation sites, and consideration of these provides further understanding of the need for benchmarking and intercalibration.

The four main assessment types are:

1. **Single point assessment**: Comparison of a measured (single or replicated) indicator value against one or more benchmarked critical values.
2. **Local spatial assessment**: Comparison of indicator measurements at different nearby locations, where conditions are otherwise similar, apart from a known gradient in N exposure.
3. **Regional spatial assessment**: Comparison of indicator measurements at widely separated locations in relation to known regional differences in N exposure, but also including other inter-site differences, such as climate, soils and other pollutant exposure.
4. **Temporal assessment**: Comparison of repeated indicator values measured over time as 'biomonitoring' in relation to temporal changes in N exposure, as well as any other changes that may have occurred.

The temporal assessment, may of course also be applied to each of the 3 assessments other assessment scales. The single point assessment at one time can only be interpreted in relation to established benchmark values. Conversely, benchmarks are not essential to examine relationships with the other three assessments, although they are still needed to determine when and where there is a significant adverse effect. These different assessment approaches are visualized in Figure 16.2, which compares the value of a given nitrogen indicator (I_N) against exposure to reactive N.

Comparison of different values with either space or time is obviously more powerful than assessment of a single value at a single time or location. Space/time assessments provide a more accurate assessment of the extent to which I_N exceeds $I_{N(critical)}$, and information on the shape of the dose-response function. Such assessments provide the basis for setting empirical critical loads for nitrogen (Sutton *et al.* 2004a), but are equally applicable for benchmarking and intercalibrating the other indicators.

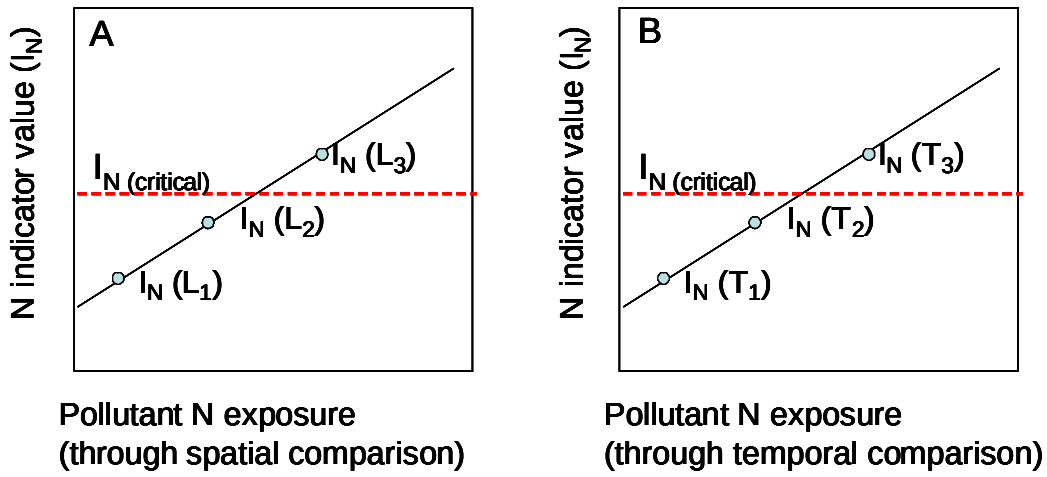

Figure 16.2. Conceptual relationship between the response of a nitrogen indicator (I_N) to pollutant N exposure. $I_{N\ (critical)}$ is the benchmark for significant effects on the ecosystem for the particular indicator used. **A**. the range of pollutant exposure is obtained through local or regional spatial comparison of different locations ($L_1..L_n$). **B**, the range of pollutant exposure is obtained by biomonitoring over time ($T_1..T_n$).

Biomonitoring of N impacts can be related broadly to three main situations:

1. **Local impacts in the vicinity of NO_x sources** and associated N emissions (including particles and NH_3). This mainly refers to roads as line sources. NO_x emissions from combustion plant may also have local direct impacts, though this has not clearly been demonstrated (due to the more dispersed nature of the emission).
2. **Local impacts in the vicinity of NH_3 sources** (in particular farms as point sources, or in transects away from agricultural land).
3. **Impacts related to diffuse sources of N deposition**, from wet deposition, plus aerosol inputs of both oxidized and reduced N. This is most relevant at locations distant from sources, so that any local gradients relate to topography rather than the emission sources.

In the first two examples, pollutant exposure varies strongly with distance from source, allowing transect studies ("local spatial assessment") to demonstrate the link between increased exposure and impacts. In these cases, it is possible to perform the dose response assessment, shown in Figure 16.2 A. This allows responses to be clearly detected and related to the pollutant source of origin. In addition, because pollutant levels may be high near sources, such studies can provide a large range of pollutant exposure, allowing clearer responses to be seen. This is particularly the case for NH_3 emissions from farms, due to the much higher local rates of N deposition this can generate compared with NO_x emissions from roads.

By contrast, for diffuse sources of N deposition, it is not possible to make a local spatial assessment in relation to nearness to source. Spatial comparisons may be made by comparing sites locally (e.g. in relation to local topographic effects on deposition) or regionally (in relation to country-scale differences in pollution levels). In such assessments for impacts of diffuse N deposition, it is however, harder to detect clear I_N signals that can be related to N exposure. This is because both topographical and regional comparisons also lead to differences in site climate, management, soils and other pollutant exposure. In addition, because diffuse sources of N deposition are necessarily already dispersed in the environment, such comparisons tend to provide a smaller range of N deposition, than in near-source assessments, making it harder to detect the I_N response. Given these increased uncertainties, the use of time as a variable for changing N exposure is particularly important for assessing the impact of diffuse atmospheric N deposition.

16.6. Defining benchmarks for different nitrogen indicators

The importance of benchmarking nitrogen indicators means that the potential to set such values is a key additional criterion in selecting practical indicator methods. Using the concept of the biomonitoring chain, Figure 16.3 suggests benchmarks in the form of different critical parameters for each indicator. The figure summarizes the status of each critical parameter on the left, while on the right, the status of model or measurement approaches to estimate the indicator is also summarized.

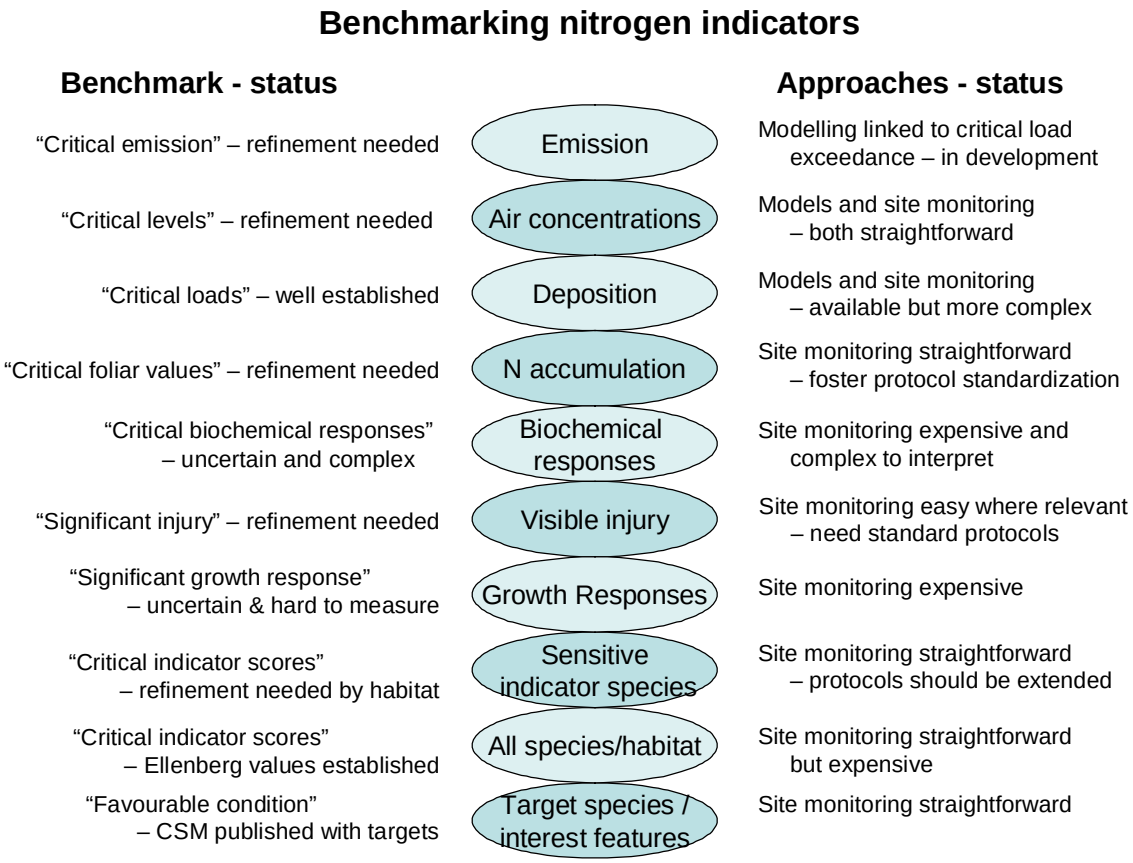

Figure 16.3. Summary of potential and existing benchmarks in relation to nitrogen indicators along the biomonitoring chain. The status of each benchmark is summarized, noting where further development of the benchmark is needed. In addition, the availability of measurement or modelling approaches is summarized for each indicator, noting the status and key development needs. Dark shaded ellipses indicate indicators that are most practical to measure/monitor.

Figure 16.3 includes some very well known benchmarks for which the approaches are well established (e.g. critical loads, CSM). However, there are many components which are uncertain and require further development.

Key points include:

- The concept of critical emission is relevant for environmental agencies. Refinement of this approach may come from the application of simple screening models, such as SCAIL (Theobald and Sutton 2003).
- While the concept of critical levels is well established, the intercalibration to critical loads and other indicators contains major uncertainties. For example, the lichen data (Section 11) and air monitoring results (Burkhardt *et al*. 1998) show that the current NH_3 critical level is not consistent with the N critical load.
- Further refinement is needed of the critical foliar values, although the emerging datasets provide a good basis for such values (e.g. Section 12).
- Biochemical responses are considered rather less certain and more complex to benchmark, given the importance of other limiting factors.
- Significant injury is an area where much has been done for other pollutants, such as O_3. Standard protocols need to be developed for the known injury signals to N.
- Growth responses of native species in the field require significant resources. Conversely, practical protocols are already available for the application of standardized grass biomonitors. Pending the refinement of benchmark values, these are best suited to local spatial assessments.
- The protocols for scoring indicator species are available, but efforts need to be placed in standardizing the critical indicator scores and range scales, both for sensitive species and for habitats.

16.7. Integrated visualisation of indicator results in relation to benchmarks

16.7.1. Integrated visualisation for hypothetical data

The integrated assessment of N indicators can support a site assessment in relation to given policy objectives, such as a review of permit for an emitting source adjacent to a European designated site. As explained above, in order to ensure a strong link between source attribution and impact, methods should be selected well-distributed along the biomonitoring chain. Given the need to be cost effective in the monitoring, the methods shown with dark shaded ellipses in Figure 16.3 provide a sound focus for measurements.

Once each indicator method is benchmarked with a critical value for a given context, it is important to compare the different indicator results in relation to the common critical values. This is illustrated by four conceptual examples in Figure 16.4, which show the percentage of the critical value for each of a series of practical indicators.

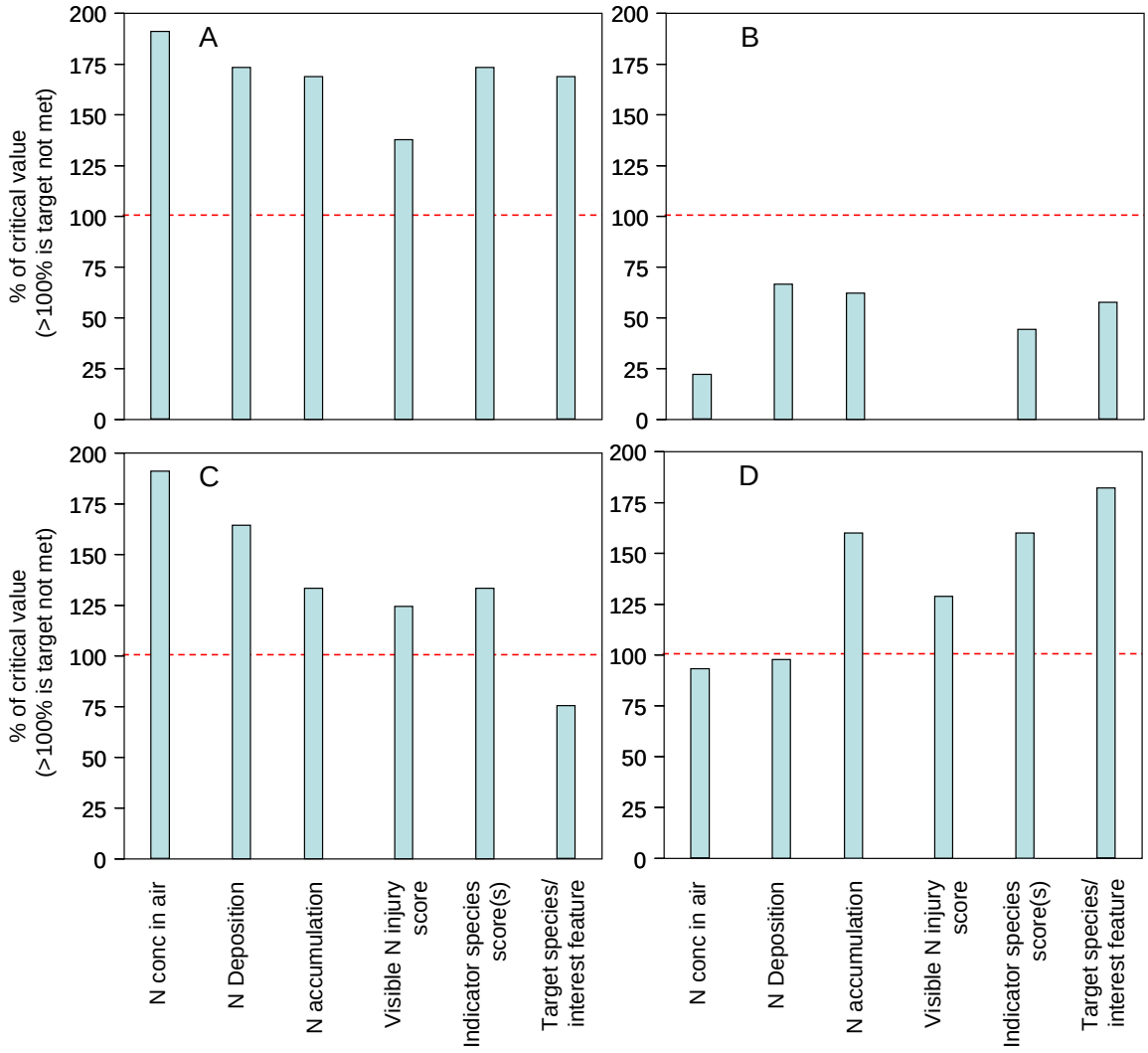

Figure 16.4. Conceptualization of how benchmarked N indicator values measured in four different contexts may be compared in relation to the critical values for each indicator.

A. Site where all indicators exceed critical values: significant effects on site integrity linked to exceedance of N exposure thresholds.
B. Site where all indicators are less than critical values: no evidence of site integrity under threat from excess N.
C. Site where interest features not recorded as under threat (e.g. CSM extent of unfavourable condition smaller than critical threshold), but all other indicators indicate a significant threat to site integrity. The decreasing profile from left to right may indicate a site of with a recent increase in N exposure, and where effects on interest features are expected in the future.
D. Site where interest features are in unfavourable condition, in agreement with results of sensitive indicator species injury and N accumulation, but where N exposure indicators are less than critical thresholds. This may reflect a site formerly exposed to high N pollution exposure, which has not yet fully recovered to favourable condition, or a site which has been subject to another stress factor (e.g. agricultural fertilization, over grazing).

Values larger than 100% reflect measurements showing that the critical value is exceeded. Should all indicators exceed 100% (Figure 16.5, Case A), this provides very strong overall evidence that a significant adverse effect is both apparent (e.g. from monitoring of interest features) and can be related to unsustainably high pollutant levels (e.g. from air concentration measurements). In addition, the intermediate indicators in the chain support the connection between source and impact. By contrast in Case B, all of the indicators are less than 100%. Hence, based on these indicator results, there is no evidence of air pollution threat and impacts in Case B.

Cases C and D illustrate how measurements distributed along the biomonitoring chain may provide additional information. In Case C, although a significant problem is not identified in the site interest feature, all the other indicators are exceeded, particularly those most closely related to N exposure. This indicates a significant adverse effect to site integrity, which may be expected to be manifested as loss of site condition in the future. Finally, case D, represents an example where exceedance of critical values for the indicators most close to the interest features could be due to either historic levels of N pollution or causes other than high nitrogen deposition. For example, such a result might be obtained if mineral N fertilizer were applied to a site.

While the four cases A to D are hypothetical and idealised, they show how the results from an integrated framework for nitrogen indicators may be visualized and interpreted. The indicator scores are presented sequentially in the order of the biomonitoring chain, while the use of intercalibrated percentage values facilitates the intercomparison between different indicators. This framework provides the basis for an operational assessment using indicator methods.

16.7.2. Preliminary benchmarking and intercalibration of nitrogen indicators

Based on the visualisation framework, the next step is to actually assign the critical indicator values and intercalibrate range scales. In a large part, this must be a major challenge for ongoing work. Nevertheless, there is already a substantial basis to set values, which allows a preliminary application of the integrated indicator framework.

The following approaches are used here to set the benchmarks and intercalibration of a selection of the most applicable indicators. These are applied in the following Section (16.7.3) to example intensive and extensive sites of this study.

Air concentrations:

Critical value: Apply the critical level for the habitat or receptor. For NO_x the annual critical level of 30 $\mu g\ m^{-3}$ as NO_2 is used here. However, for NH_3 it is known that the critical level for NH_3 (8 $\mu g\ m^{-3}$; annual mean) is not well intercalibrated with the critical load (Burkhardt *et al*. 1998). This is acceptable if application of the critical level is solely to reflect direct concentration effects, as compared to the consequences of NH_3 on N deposition. However in the present context, the aim is to intercalibrate a baseline of minimum significant effect. Therefore, it is relevant here to consider the NH_3 air concentration that would give rise also to indirect effects (which may be much lower than 8 $\mu g\ m^{-3}$). Additionally, the lichen data from the present study (Sections 7 and 11) and those of Sutton *et al*. (2004a) point to direct effects of NH_3 at mean concentrations of 1-2 $\mu g\ m^{-3}$. For the present application, the critical value for NH_3 is set conservatively at 2 $\mu g\ m^{-3}$.

Range scale: For concentrations (C_N), this is straightforward and linear, with the % value being derived as $C_{N\ measured} / C_{N\ critical\ value} * 100$.

Deposition:

Critical value: This is the best established nitrogen indicator, through the use of critical loads. Empirical values are available for a wide range of habitats (Achermann and Bobbink 2003) and can be applied directly. In the preliminary examples below, the results are calculated using just two nominal values of the critical load: 15 kg N $ha^{-1}\ y^{-1}$ for woodland sites and 10 kg N $ha^{-1}\ y^{-1}$ for sites containing both woodland and bog habitats (Brown Moss, Castle Ennigan).

Range scale: For deposition (D_N), the range scale is straightforward and linear, with the % value being derived as $D_{N\ estimated} / D_{N\ critical\ load} * 100$.

Moss total foliar nitrogen content:

Critical value: This is probably the best established measured bioindicator for N impacts. Significant attention has already been given to establishing critical values (e.g. Pitcairn *et al*. 2002) and these are also estimated in previous chapters of this report both as an average (1.3% tissue N) or habitat specific values. In the preliminary examples below, a standard critical value of 1.3% is applied.

Range scale: The response of total foliar nitrogen (F_{TN}) to atmospheric nitrogen deposition is not 1:1 proportional and therefore the range scale needs to be calibrated. An attractive approach is to apply the results of the regression between foliar N and deposition, thereby providing an estimated N deposition input based on the bioindicator. This has the advantage that the result can be scaled directly against the critical load. However, a reasonable proportionality can be obtained for F_{TN} with the following simpler scaling, which is used in the examples, the % critical value being $(F_{TN\ observed})^2/(F_{TN\ critical})^2 *100$.

Moss soluble ammonium concentration:

Critical value: Based on the revised extraction protocol reported in Appendix I, an average critical value of this parameter has been assessed as 6 μg NH_4^+-N g^{-1} FW (Section 13). Although, Section 13 investigates the use of habitat specific values, to main simplicity in the following preliminary examples, this single value is applied.

Range scale: The response of soluble foliar ammonium content ($F_{NH4\text{-}N}$) to atmospheric N deposition has been clearly shown to have a much stronger slope than that to F_{TN} (Sutton *et al*. 2004a, this report (Section 6). Again the regression between deposition and $F_{NH4\text{-}N}$ might be used as a predictor of atmospheric N deposition with results scaled against critical loads, but this requires further development. For the following examples, a simpler scaling is used whereby the % indicator value is $(F_{NH4\text{-}N\ observed})^{0.5}/(F_{NH4\text{-}N\ critical})^{0.5} *100$, which is found to give broad intercalibration with the F_{TN} values.

Lichen acidophyte-nitrophyte scores

Critical value: As with the moss chemistry methods, two approaches may be considered, either using critical lichen values directly, or by relating a critical lichen value to the critical air concentration. In the former (not used in the following examples), the data from the UK extensive survey (Section 11) indicate a critical value for the difference between the acidophyte and nitrophyte scores (AV-NV) of +5 for trunks and 0 for twigs, with smaller values indicating exceedance. This approach may be useful where there is no data available on bark pH. In the approach applied in the following examples, the critical value is related through regression from the UK survey results (Section 11) to a critical NH_3 concentration, which is taken in this instance as 2 μg m^{-3}.

Range scale: Where only the AV-NV score is available, without data on bark pH, the following range scales provide a reasonable intercomparability between the twig and trunk lichen data (acid barked species, such as oak, only): For trunks the % indicator result may be calculated as (NV-AV+17)/7 * 100. For twigs comparable values may be calculated as (NV-AV+5)/5 *100.

It has been shown however, in Section 11 that there is a significant interaction between bark pH, atmospheric ammonia concentration and the AV-NV score. To account for this the multiple regression of AV-NV vs. bark pH and NH_3 concentration may be transformed to provide a 'calculator' for NH_3 concentration (μg NH_3 m^{-3}). Based on the lichen estimates of NH_3 concentration ($C_{NH3(lichen)}$) the % indicator can be taken as $C_{NH3(lichen)\ observed}$ / $C_{NH3\ critical}$ * 100.. The same formula can be applied for twigs and trunks:

$$C_{NH3(lichen)} = 23.63 – 0.615(AV\text{-}NV) – 4\ (bark\ pH)$$

This approach is used in the following examples for lichen indicators. It is important to note that this function is limited to low NH_3 concentrations (<8 μg m^{-3}), since at very high NH_3, the positive effect of NH_3 on bark pH becomes important compared with the differences in bark pH due to different tree species. This limitation may be assessed by using a critical bark pH value appropriate for different tree species and specific for twigs and trunks. Hence uncharacteristically large bark pH values provide a flag for very high NH_3 concentrations, showing that this equation is not applicable.

Ellenberg scores for higher plants and mosses:

Critical value: There currently seems to have been little focus on benchmarking Ellenberg scores and much more effort is required. For the present preliminary application in the following section only a woodland example is considered. In this case, from the transect studies away from point sources, a critical unweighted Ellenberg mean value of 5 is applied, and this would be expected to vary between habitats.

Range scale: Setting the range scale for mean Ellenberg score (E_N) is equally challenging, particularly since the relationship with atmospheric deposition is so variable. For the present preliminary estimate, the following approach to calculate the Ellenberg % critical indicator is broadly comparable with the other indicators: ($E_{N\ recorded}$ + 1 - $E_{N\ critical\ value}$) * 100. Using a critical value of 5 means that a recorded mean E_N score of 5 gives 100%, while a recorded value of 7 gives 300%.

16.7.3. Integrated visualisation for actual data from this study

The results of applying these benchmark and intercalibrations to example results from this study are shown in Figure 16.5.

The clearest pictures emerge for the Castle Ennigan and Ariundle sites. For Castle Enigan, all the nitrogen indicators indicate exceedance of the benchmark values. From the available parameters, it can be fairly concluded that NH_3 is a major threat to the Castle Ennigan site with altered integrity in terms of moss chemistry and lichen species composition. Actual change in the species composition of the designated features was not assessed, but significant adverse effects would be expected. By contrast, for Ariundle, none of the nitrogen indicators exceed the benchmark values, indicating that this site is not under significant threat from nitrogen deposition.

Of the examples in Figure 16.5, Brown Moss is also shown to be under substantial threat from nitrogen deposition. All the indicators exceed the benchmarks, with the exception of just one (moss ammonium content). Again, although actual change in designated species composition was not assessed, significant adverse effects are expected.

While Castle Enigan and Brown Moss show sites with significant problems due to NH_3, these may be contrasted with Borrowdale and Yarner Wood, where NH_3 levels are low, but N deposition still exceeds critical loads, implying sites with high wet N deposition. It is interesting to note that at both Borrowdale and Yarner Wood the N deposition is the only indicator that exceeds the critical values. This does not mean that significant adverse effects are not expected, but simply that the bioindicators used have failed to demonstrate such effects, and that a more comprehensive monitoring program might still detect effects (e.g. through assessments methods 2, 3 or 4, Section 16.5). Borrowdale provides a good example of this: although the moss foliar N values do not exceed the critical values, a detailed study of growth and foliar N using bryophytes reciprocally transplanted between Ariundle and Borrowdale showed substantial significant N effects at Borrowdale (increased foliar N and reduced growth rates) (Mitchell *et al.* 2004).

It may be noted that at Yarner Wood and Ariundle, some negative indicator values are shown, which is theoretically not possible. This is simply due to scatter in the results, particularly where the indicator value is derived by regression with atmospheric deposition or concentration.

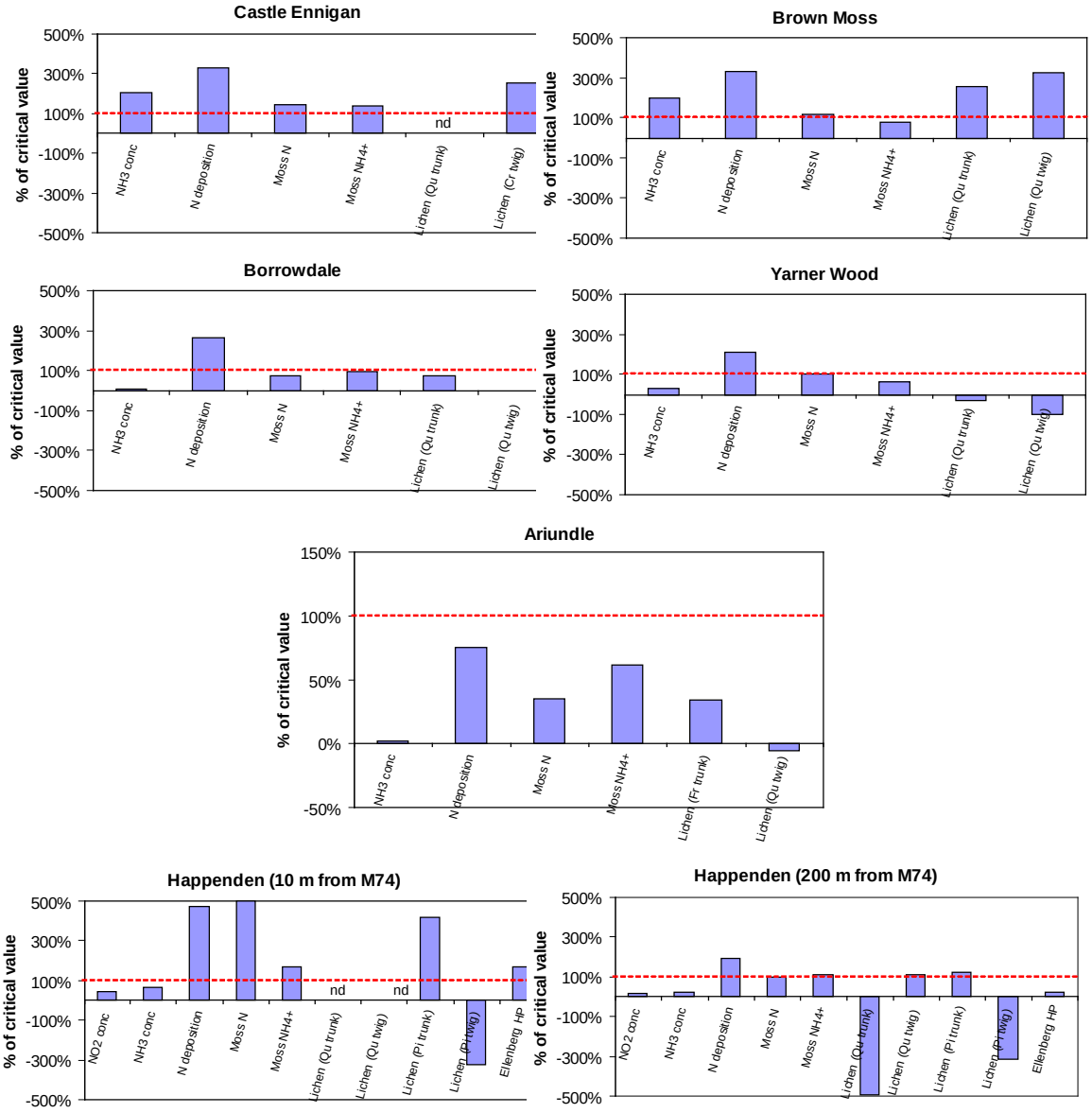

Figure 16.5. Examples of benchmarking nitrogen indicators at sites from this study. Note the different scale for Ariundle; nd = not determined.

The last example of Happendon Wood provides the most complex picture to interpret, and for this purpose the local spatial comparison between results 10 m from the M74 motorway with those 200 m from the M74 is useful. Overall, higher values are shown at the site closest to the motorway. Thus the impact of the motorway on the 10 m site is clear. However, there is a large amount of scatter in the bioindicator results and wide variation between the different indicators. This almost certainly reflects the fact that responses to road pollution are different to those from NH_3 from agriculture. This point was emphasised by the standardised grass biomonitor results (see Section 5), which recorded a *decrease* in growth adjacent to the motorway, despite increased N inputs. Another explanation is that the response to the road is modest compared with the very

high wet deposition inputs at this site (shown by the distinction between NH_3 and NO_x concentrations versus total N deposition). Even at the 200 m site, moss chemical parameters are already at the critical limit, although these are increased substantially by the presence of the road at the 10 m site for both total N and foliar ammonium. At the two Happendon Wood sites the lichen assessment gives rather unclear results, and this may be explained by three reasons: a) data for oak were not available for the 10 m site and the 200 m site shows significant scatter, but no clear effect, b) the remaining data are for pine, for which the lichen methodology is much more uncertain, c) the influence of roads and associated N on lichens is significantly different to that of NH_3 from agriculture.

A detailed Ellenberg assessment of the woodland ground flora was made at the Happendon Wood sites. This showed that while the site most distant from the motorway did not exceed the Ellenberg benchmark, the site adjacent to the motorway exceeded it substantially.

16.8. Conclusions

It is obvious that there is significant work needed to refine the intercalibration of nitrogen indicators. In addition to benchmarking the indicators, with critical thresholds, it is essential to ensure that the quantitative responses are comparable. This may seem obvious, but it actually requires careful design of the indicator scales to ensure comparability between such a wide range of different data types (e.g. concentration, deposition, foliar concentrations, injury, species diversity scores etc) and responses.

The choice of how to set such scales can be informed by considering the purpose of site assessments with bioindicators (c.f. Figures 16.4 and 16.5). Firstly, these focus on establishing an integrated quantification of the status of the system in relation to the benchmarks. Secondly, they provide a framework to monitor the success of policy and management actions to reduce atmospheric N deposition to sustainable levels. Given the importance of this second objective, the indicator scales should, as far as possible be set in proportion to the extent of pollutant exposure. Hence, in the example of Figure 16.4a, a reduction of current pollutant exposure of at least 75/175 = 43% would be required to bring the indicators within the critical values in the long term. As noted, scaling indicators by exposure also has the practical advantage in that the scales for air concentrations and N deposition are already well established ($\mu g\ m^{-3}$ and $kg\ N\ ha^{-1}\ y^{-1}$, respectively), with the critical values (critical levels and critical loads) being widely recognized.

Two elements to indicator the range calibration need to be addressed. The first is the *response intercalibration* of the indicators as normally measured. In this case, the objective is to relate quantitatively the response of one indicator to another, e.g. a lichen diversity indicator score to given NH_3 air concentrations. The second is the *unit normalization* of the indicator scores, so that measured values for different indicators can be interpreted on a common basis. In this case, the basic indicator value is transformed to a related measure and presented as a percentage of the critical indicator value in a way that aims broadly to maintain the proportionality between indicators for values above and below 100%.

Recommendations for future work

The following key recommendations are identified:

- The current study found that the selected bioindicator methods were robust at sites with defined N point sources. However, for sites with diffuse long-range N deposition it was more difficult to quantify the impacts of N. Therefore, further development of bioindcator methods needs to be targeted at sites with long-range and often wet dominated N deposition.

- The successful testing of epiphytic acidophyte and nitrophyte macrolichen indicator species against atmospheric pollution and environmental data will provide a basis for the development of a standardised method for widespread use in the UK. Therefore, there is a requirement to refine the simplified protocol and provide an illustrated guide for use by field officers either as part of the CSM or specifically targeted at sites identified as potentially at risk from atmospheric N impacts.

- The new acidophyte/nitrophyte index for vascular plants and bryophytes identified in the Ellenberg study of intensive sites requires further refinement using key species. The development of this approach using the sensitivity of key species in response to nitrogen requires a limited botanical training, could be a useful addition tool for site staff (including conservation and environmental agency staff). Analysis of existing datasets and additional field survey work are required to refine this approach.

- Bioindcators can provide an 'early warning' signal of potential N impacts to designated nature conservation sites. Establishing the response timescale to the 'end point signal' is also an important feature in using such indicator species. The use of bioindicators and their appropriate timescale in response to exposure requires testing across sites at risk from N deposition by regular monitoring using selected biomonitoring methods following specific examples of known changes in N deposition.

- The use of the biochemical method for detecting tissue soluble ammonium concentration proved to be a useful additional biomonitor tool for pleurocarpous mosses. However, further refinement of this method is required using an extended range of moss species in order to provide an index of the relationship between soluble ammonium concentration and atmospheric NH_3 concentration and N deposition.

- The UK extensive study showed that pleurocarpous mosses responded to changes in N and particularly NH_3-N concentrations. As NH_3 concentrations are very site specific, the relationship between moss foliar N concentrations and NH_3 should be further refined by sampling selected pleurocarpous moss species at the National UK Ammonia Network sites.

- The UK extensive study has shown that there are moss species-specific responses to both NH_3 concentrations and N deposition. Therefore, screening of the common moss species to determine their individual response to both NH_3 and N deposition is required. It is also recognised that there is seasonal variation in N concentrations in pleurocarpous mosses. This variation may be species-specific and repeated seasonal measurements at key sites.

- The current study identified that a defined pleurocarpous mosses tissue N content and soluble ammonium concentration could be determined for specific habitats. This was based on a limited number of sites and habitats. Additional work is required to further refine the N concentrations for those habitats already identified and to also include additional habitats.

- The UK scale lichen assessment provided a remarkable new dataset demonstrating the sensitivity of lichen responses to NH_3 at a UK scale and the interactions with bark pH. The method is so far based only at 32 sites, and should be extended by further testing at other locations across the UK. In particular, use of the other sites in the National Ammonia Monitoring Network could allow the calibration to be improved by recording at a further 50+ sites (depending on local availability of suitable trees).

- The lichen assessment highlights the increased sensitivity of lichens on twigs compared with those on trunks of the same tree species, linked to the differences between bark pH.

It has been suggested that there is an association with the age of the substrate and that lichen communities of the trunk may carry relicts of former conditions while colonisers of the new twig substratum are associated with present conditions. This expectation needs to be properly tested by sampling at sites following a known perturbation (e.g. monitoring change after the installation of a new farm source).

- This report has also provided a theoretical analysis of approaches to improve robustness in biomonitoring and the applicability of results. It is evident that further effort needs to be placed in refining the benchmarking and intercalibration of indicators in order to refine an integrated operational approach to nitrogen indication.

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Appendix I: Development of methods for the measurement of foliar soluble nitrogen as a bioindicator of atmospheric nitrogen deposition

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A1.1. Introduction

As with tissue N content, soluble N concentrations of foliage can be used as indicators of atmospheric N deposition and its ecological impacts. Firstly, total soluble N concentration of foliage may be measured, which provides an estimate of "substrate nitrogen" (Riedo *et al*. 2002), which is the nitrogen that is directly available for incorporation into new carbon compounds. Secondly, key soluble components may be measured, such as amino acids, total foliar ammonium or total foliar nitrate. Although, extensive research has been done on a number of amino acid compounds (Näsholm *et al*.1994), recent studies of Sutton *et al*. (2004a) indicate that foliar ammonium provides a good integrator of freely available nitrogen in the plant. Physiologically, this may be explained by the important role that ammonium plays as a substrate for protein and other organic-N compound synthesis, and a key product of proteolysis. Increased nitrogen availability in plants may thus be reflected in increased cycling and pool size of foliar ammonium. Strictly, there are many different foliar ammonium pools, such as in the apoplast versus the symplast. However, as symplastic ammonium concentrations tend to be much larger than those of the apoplast, the total or bulk concentration provides an indicator which is most close to the symplastic value. Conversely, as measurement of bulk concentrations is much easier than symplastic or apoplastic concentrations, the bulk value is more suited as a practical nitrogen bioindicator.

The limited available data suggest that the relationship to atmospheric N deposition is more precise (or can be measured more precisely) for foliar NH_4^+ than for substrate N (Sutton *et al*. 2004a). At present there is requirement for more data to make the link between these parameters and plant community changes. In Phase 1 of the bio-monitors study it was found that measurement of soluble N as foliar ammonium (NH_4^+) could be used as a sensitive chemical bioindicator of N in pleurocarpous mosses (Sutton *et al*. 2004a; Leith *et al*. 2003).

Our standard extraction method for soluble N, adapted from Loubet *et al*. (1999, 2002), requires plant material to be ground in liquid nitrogen. Although this method effectively breaks down the cell structure, it is time consuming.

The aim of this study was therefore to develop and validate a quicker, simpler and cheaper extraction method with no use of liquid nitrogen.

A1.2. Methods

A1.2.1. Tests performed

Several tests compared a range of extraction methods and the effects of environmental conditions and plant species. The tests were focused on the extractions for different moss species and a comparison also made with selected grass species.

The following series of tests were made:

Test 1: The effect of extraction time in ultra-sonic extraction.

Test 2: Comparison of several different extraction methods: extraction in liquid nitrogen compared with extraction in water, extraction in acid, use of autoclave, use of an ultra-sonic bath and different extraction times.

Test 3: Water extraction procedure: influence of temperature, light and 'leaking time' on concentration.

Test 4: Water extraction procedure: comparison for different moss species.

The emphasis of the tests was the measurement of foliar ammonium concentrations, as this appeared from previous measurements (Sutton *et al*. 2004a, Leith *et al*. 2003) to have the best potential. However, selected comparisons were also made with the measurement of total soluble nitrogen and of total foliar nitrate. In relation to the last, a question of interest in application of the method was how foliar ammonium and foliar nitrate would respond to changes in reduced and oxidized nitrogen deposition.

A1.2.2. Sample preparation (general)

All the plant material used was sorted and cleaned as soon as possible after harvest: only the green parts were used and all soil, leaf bits etc were removed. The cleaned sample was quickly rinsed with de-ionised water, blot-dried with tissue paper and stored in the freezer at -20°C until the samples were prepared for the different extraction methods.

A1.2.3. Calculation of concentration

For each of the extraction procedures tested, the concentration of soluble N, NO_3^- and NH_4^+ are calculated as:

$$N_{f,x} = [N_x] * (M_{extractant} + M_{f,sample}) / M_{f,sample} \quad (1)$$

where $N_{f,x}$ is the foliar nitrogen concentration for the relevant nitrogen component x (μg g^{-1} fresh weight), i.e. total soluble nitrogen ($N_{f,sol}$), total ammonium ($N_{f,NH4+}$) or total nitrate ($N_{f,NO3-}$), $[N_x]$ is the aqueous phase concentration of the nitrogen component in the extraction solution (μg g^{-1}), $M_{extractant}$ is the mass of extractant used (10 g as standard) and $M_{f,sample}$ is the fresh mass of the leaf sample (g).

A1.3. Test 1: The effect of extraction time in ultra-sonic extraction.

In this test plant material in a de-ionised water solution was placed in a Sonic bath (ultrasonic vibrations) for different time periods to test the sensitivity of the method to extraction time.

This test provided the basis for comparing the sonication extraction method with other extracting methods.

A1.3.1. Material and method

Plant material
A grass, *Lolium multiflorum* and a pleurocarpous moss species, *Hypnum jutlandicum* were used for this test. Not treated *Lolium multiflorum* plants from the 'evaluating *Lolium multiflorum* as a standardised grass bioindicator for gaseous ammonia' (Appendix II) were used. *Hypnum jutlandicum* was collected from Whim Moss background ambient (no treatment).

Sample preparation and chemical analysis
Fresh plant material was collected from and prepared according to Section A1.2.2. For each sample for this test, 1 g of frozen plant material was put in a 20 ml plastic bottle (polyvial®v) and 10 ml of de-ionised water was added. The samples were then placed in the ultra-sonic bath for different time periods (see below). After sonication times the solution was filtered (PURADISC ™ 25PP disposable filter, 0.45µm Polypropylene), divided in 3 sub samples of 3 ml and stored in a -20°C freezer until chemical analysis.

The respective 3 ml sub-samples were analyzed for:

a) soluble NH_4^+ by AMFIA (Ammonia Flow Injection Analysis)
b) soluble NO_3^- by IC (Ion Chromatography)
c) soluble N by ANTEK (Chemiluminescent Nitrogen Detector)

Due to the small amount of sample solution available, there was only one sub-sample for each analysing method.

Extraction time in sonic bath
The samples were extracted in the ultra-sonic bath for 0, 0.5, 2, 8, 32 and 128 minutes. After the samples have been taken out of the sonic bath they stood for 2.5 hours at room temperature. This was called 'leaking time', to allow the NH_4^+, NO_3^- and N to 'leak' out the plant material into the sample solution. Additionally, there was an extra sample for 128 minutes extraction time, but the leaking time for this sample was 24 hours.

A1.3.2. Results

Table A1.1 shows the estimated concentrations in the plant material ($N_{f,x}$) for soluble NH_4^+, NO_3^-, and N according to the different extraction times. These results are shown graphically in Figures A1.1 and A1.2.

Table A1.1. Concentrations of soluble NH_4-N, NO_3-N and total soluble N in ($\mu g\ g^{-1}$ F.W.) in *L. multiflorum* and *H. jutlandicum* after extracting the plant material in the ultra-sonic bath for different times.

Plant species	Extraction time (min)	Leaking time (hours)	$N_{f,NH4+}$ ($\mu g\ N\ g^{-1}$ FW)	$N_{f,NO3-}$ ($\mu g\ N\ g^{-1}$ FW)	$N_{f,sol}$ ($\mu g\ N\ g^{-1}$ FW)
L. multiflorum	0	2.5	15.1	4.04	1071
	0.5	2.5	12.1	3.01	1318
	2	2.5	12.9	3.79	1490
	8	2.5	13.4	3.56	1453
	32	2.5	13.5	2.54	1452
	128	2.5	18.7	3.61	2561
	128	24	45.7	n.d.	2838
H. jutlandicum	0	2.5	70.1	0.58	101567
	0.5	2.5	63.2	0.38	102102
	2	2.5	64.1	0.48	105705
	8	2.5	68.0	0.39	106974
	32	2.5	69.1	0.42	105954
	128	2.5	112.9	0.48	241043
	128	24	140.1	n.d.	234317

n.d. = not detectable

The *H. jutlandicum* had a much higher N_f *Hypnum jutlandicum*$_{,NH4+}$ compared with the grass *L. multiflorum.* This was also found for the moss species tested in Phase I of the project (Sutton *et al*. 2004a)

The values of $N_{f,NO3-}$ were much lower than those for $N_{f,NH4+}$ especially in the *H. jutlandicum*. In addition, $N_{f,NO3-}$ was not detectable after a 24 hours 'leaking time', which may be an indication that NO_3^--N is not stable in the sample solution.

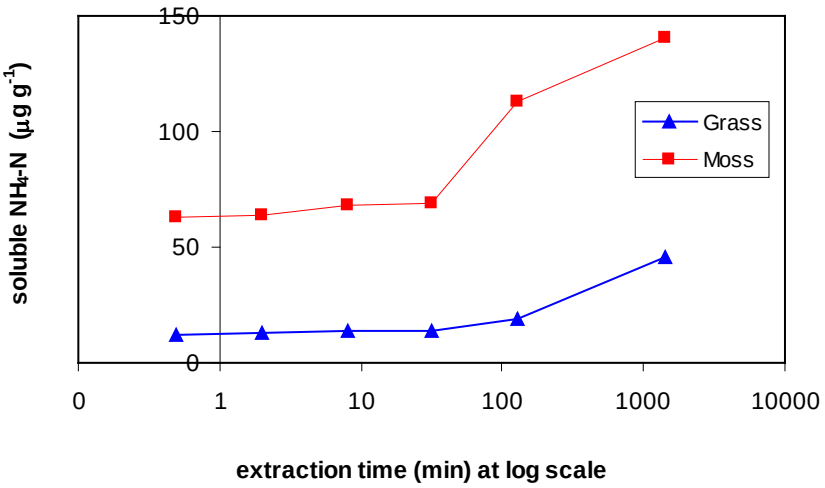


Figure A1.1. Measured concentration of soluble NH_4-N (µg g^{-1}) in moss (*Hypnum jutlandicum*) and grass (*Lolium multiflorum*) using a sonification extraction that avoids the grinding of plant material. Using the sonification method an extraction period of at least 1000 minutes (inclusive leaking time) is necessary.

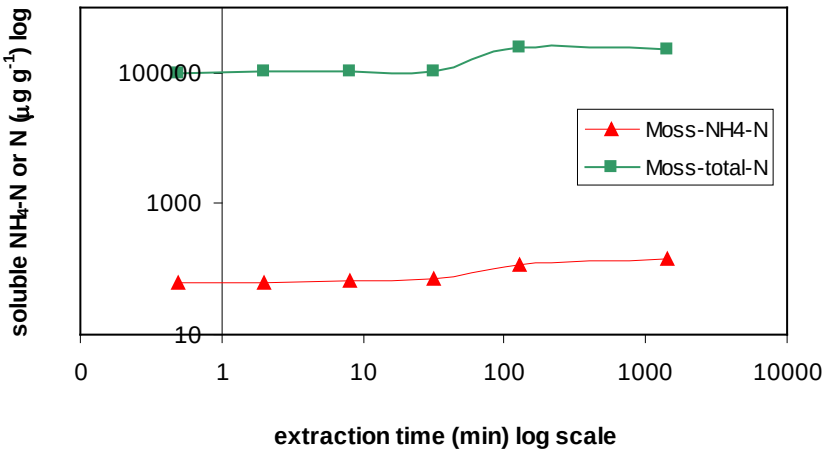


Figure A1.2. Measured concentration of soluble NH_4-N and N (µg g^{-1}) in moss (*Hypnum jutlandicum*) using a sonification extraction that avoids the grinding of plant material

Table A1.1 and Figures A1.1 and A1.2 show that the sonication method extracts more soluble N with increasing sonication times for $N_{f,sol}$ and $N_{f,NH4+}$. By contrast, there was no significant increase in $N_{f,NO3-}$ for increasing extraction periods (Table A1.1), with the largest values actually being for a water only extraction, with no sonication.

On the basis of these tests, it was concluded that in further tests the plant material in de-ionised water should be sonicated for 2 hours before the soluble N is extracted. The reason for this was that this provided a larger extract than for shorter periods, which were stable over periods of between 8 to 32 minutes, but with smaller values. These results also make it clear that the 'leaking time' (time after sonication treatment to allow the NH_4^+ to 'leak' out of the plant material into the solution) is important.

A1.4. Test 2: Comparison of several different extraction methods

In Test 2 different extraction methods were compared. The main methods tested were:

a) sonication,
b) autoclaving
c) effect of acid in the extractant, and
d) the adapted liquid nitrogen method of Loubet *et al*. (1999, 2002) with variations in the extraction time and the substrate used.

A1.4.1. Material and Methods

Plant material
The pleurocarpous mosses (*Pleurozium schreberi* and *Hypnum jutlandicum*) were collected from a *Calluna vulgaris* dominated bog at the CEH Whim Moss NH_3 field release study (Section 3.6). NH_3 is automatically released from a 10 m line source along a 60 m transect of the bog when meteorological conditions are met. *P. schreberi* was collected at the following distances along the transect from the NH_3 line source: 9, 27 and 60 m and *H. jutlandicum* at 9, 60 m and at background ambient (control). The mean NH_3 concentrations in the air along the transect at 9, 27, 60 m and ambient background are 95, 22, 5 and 0.5 μg m^{-3} respectively. The *Lolium multiflorum* was grown from seed in the CEH glasshouses as part of the grass biomonitors experiment (Appendix 2). The plant material was cleaned, washed in de-ionised water and stored in a -20^0C freezer until extracted and analysed.

Extraction methods.
The following extraction methods were tested:

1: water, leaking for 24 hours

2a: sonic bath 2 hours, no leaking

2b: sonic bath 2 hours, leaking for 24 hours

3a: autoclave H_2O, 10 min 110˚C, 3 hours cooling down, no leaking

3b: autoclave H_2O, 10 min 110˚C, 3 hours cooling down, leaking for 24 hours

4a: autoclave H_2SO_4, 10 min 110˚C, 3 hours cooling down, no leaking

4b: autoclave H_2SO_4, 10 min 110˚C, 3 hours cooling down, leaking for 24 hours

5a: liquid N, no leaking

5b: liquid N, leaking for 24 hours

In method 4, a dilute concentration (0.1 M) of H_2SO_4 which acts to conserve NH_3, was used to check if any NH_4^+ was lost in the autoclaving process through volatilisation. Method 5 is the soluble N extraction method used to date and is the 'standard method'. This procedure involves grinding the plant samples in liquid nitrogen using a mortar and pestle. **Leaking** is defined as allowing the sample (in de-ionised water) to stand for a defined period after sonication/autoclaving treatment prior to chemical analysis.

Sample preparation
1 g of frozen plant material was put in a 20 ml plastic bottle (polyvial®v) and 10 ml of de-ionised water was added. Three replicates of each sample were prepared where possible, but there was not always enough plant material. The different extraction procedures were performed, after which the solution was filtered (PURADISC ™ 25PP disposable filter, 0.45μm Polypropylene), divided into 3 x 3 ml sub-samples and stored at -20^0C until analysed for:

a) soluble NH_4^+ by AMFIA (Ammonia Flow Injection Analysis)
b) soluble NO_3^- by IC (Ion Chromatography)
c) soluble N by ANTEK (Chemiluminescent Nitrogen Detector)

To test if NH_4^+ was lost or added during the extraction time, some blank solutions (de-ionised water) and some solutions with a known amount (1 ppm or 10 ppm) of NH_4^+ were tested. These solutions were frozen immediately (control), 2 hours (method 2a) or 3 hours (method 3a and 4a) and 24 hours (method 2b, 3b and 4b) after preparation and kept in the freezer until analysed for NH_4^+ by AMFIA.

The influence of the filtering process on the concentration of NH_4^+ was tested, although previous work had shown that filtering samples did not have an influence on the NH_4^+ concentrations in aqueous solutions.

A1.4.2. Results

The results of the different extractions for *L. multiflorum*, *P. schreberi* and *H. jutlandium* are shown in Tables A1.2, A1.3 and A1.4 respectively as well as in Figures A1.3, A1.4 and A1.5.

Table A1.2. Soluble NH_4-N, NO_3-N and total N concentrations in *Lolium multiflorum* using different extraction methods.

Method	**$N_{f,NH4+}$ (µg g^{-1} FW)**	**$N_{f,NO3-}$ (µg g^{-1} FW)** [a]	**$N_{f,sol}$ (µg g^{-1} FW)**
1	79.3	272	1086
2a	21.1	292	1331
2b	27.6	555	1543
3a	37.0	897	n.d. [c]
3b	42.7	1010	n.d. [c]
4a	125.0	n.d. [b]	n.d. [c]
4b	121.0	n.d. [b]	n.d. [c]
5a	12.3	767	976
5b	73.9	3857	1533

Notes: **a**, NO_3-N concentrations not reliable because they were far out of the calibration range of the used standards. There was not enough sample solution left to repeat the measurements with diluted samples. **b,** concentrations were not determined (n.d.) due to sulphate interference in the ion chromatographic analysis of nitrate (Method 4), **c**, samples not determined (n.d.) due to failure of the ANTEK chemical analysis system.

The NO_3-N measured concentrations in this *Lolium multiflorum* test are not reliable because they were far out of the calibration range of the used standards. The correct concentrations are probably far lower than the measured ones. There was not enough sample solution left to repeat the measurements with diluted samples.

Table A1.3. Soluble NH_4-N, NO_3-N and total N concentrations in *Pleurozium schreberi*, collected at different distances from a NH_3 source at Whim Moss, using different extraction methods.

Distance from source (m) (mean NH_3 conc, ug m^{-3})	**Method**	**$N_{f,NH4+}$ (µg g^{-1} FW)**	**$N_{f,NO3-}$ (µg g^{-1} FW)**	**$N_{f,sol}$ (µg g^{-1} FW)**
9 (95 µg m^{-3})	1	112.9	9.4	714
9	2a	69.3	13.0	1269
9	2b	79.1	5.0	1130
9	3a	105.4	10.9	n.d. **b**
9	3b	111.7	11.2	n.d. **b**
9	4a	159.2	n.d. **a**	n.d. **b**
9	4b	176.9	n.d. **a**	n.d. **b**
9	5a	40.6	4.0	508
9	5b	92.6	25.3	947
27 (22µg m^{-3})	1	127.6	1.4	872
27	2b	107.8	13.6	1278
27	3b	139.7	8.2	n.d. **b**
27	4b	202.2	n.d. **a**	n.d. **b**
27	5a	55.8	5.4	745.
60 (5 µg m^{-3})	1	25.1	4.3	742
60	60	21.1	16.6	770
60	3b	32.3	8.80	n.d. **b**
60	4b	53.3	n.d. **a**	n.d. **b**
60	5a	5.9	7.1	486

Notes: **a**, concentrations were not determined (n.d.) due to sulphate interference in the ion chromatographic analysis of nitrate (Method 4), **b**, samples not determined (n.d.) due to failure of the ANTEK chemical analysis system.

Table A1.4. Soluble NH_4-N, NO_3-N and total N concentrations in *Hypnum jutlandicum,* collected at different distances from an NH_3 source at Whim Moss, using different extraction methods.

Distance from source (m) (ug m-3 NH_3)	**Method**	**$N_{f,NH4+}$** ($\mu g\ g^{-1}$ FW)	**$N_{f,NO3-}$** ($\mu g\ g^{-1}$ FW)	**$N_{f,sol}$** ($\mu g\ g^{-1}$ FW)
9 m (95 ug m^{-3})	1	300	1.42	660
	2b	233	12.7	1043
	3b	267	6.91	n.d. **b**
	4b	596	n.d. **a**	n.d. **b**
	5a	118	5.77	536
60 m (5 ug m^{-3})	1	23.9	4.31	705
	2b	32.3	14.4	764
	3b	37.8	7.76	n.d. **b**
	4b	64.4	n.d. **a**	n.d. **b**
	5a	5.60	8.60	367
Ambient (0.5 ug m^{-3})	1	11.7	7.43	8.71 ??
	2a	11.5	10.8	449
	2b	17.5	11.3	493
	3a	21.1	10.3	n.d. **b**
	3b	21.3	10.3	n.d. **b**
	4a	37.8	n.d. **a**	n.d. **b**
	4b	36.1	n.d. **a**	n.d. **b**
	5a	0.99	8.45	149
	5b	10.4	19.5	304

Notes: **a**, concentrations were not determined (n.d.) due to sulphate interference in the ion chromatographic analysis of nitrate (Method 4), **b**, samples not determined (n.d.) due to failure of the ANTEK chemical analysis system.

Table A1.5. N content (% N) of the plant material (grass and moss) used in test 2: Different extraction methods.

Species	Distance from NH_3 source (m)	% N
L. multiflorum	-	1.48
H. jutlandicum	9	1.81
H. jutlandicum	60	1.30
H. jutlandicum	Ambient	1.11
P. schreberi	9	1.88
P. schreberi	27	1.95
P. schreberi	60	1.46

P. schreberi has a slightly higher N content than *H. jutlandicum*.

a) Extraction Methods

Hardly any loss or addition of NH_4^+ was found in the standard solutions during the extraction or filtering process (Tables A1.6-A1.9).

Table A1.6. Measured NH_4^+ concentration before and after filtering the NH_4^+ standard solution. Concentration is the mean of 3 measurements.

Standard NH_4^+ concentration (µg g^{-1})	Filtered solution		Not filtered solution	
	Measured Concentration (µg g^{-1})	Standard deviation	Measured Concentration (µg g^{-1})	Standard deviation
1	0.998	0.013	1.000	0.003
10	10.026	0.018	10.058	0.016

Table A1.7. Measured NH_4^+ concentration of standard NH_4^+ solutions after 'extraction' in the sonic bath. Concentrations are the mean of 3 or 5 measurements.

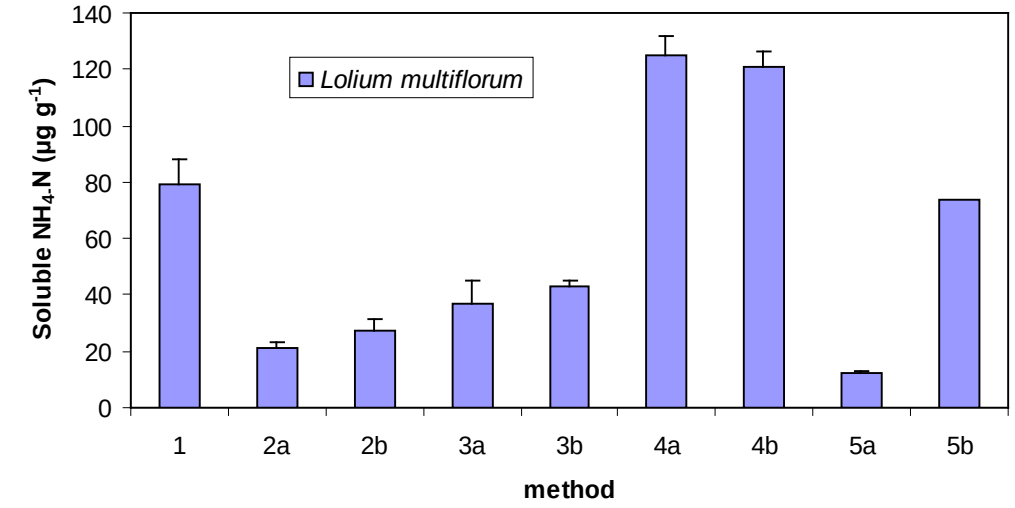
NH_4^+ concentration in standard (µg g^{-1})	Extraction method	Measured NH_4^+ concentration (µg g^{-1})	Standard deviation
0	Control	0.010	0.005
0	2a	0.009	0.001
0	2b	0.009	0.002
1	Control	0.997	0.006
1	2a	0.996	0.006
1	2b	0.993	0.005
10	Control	10.061	0.040
10	2a	10.102	0.048
10	2b	10.114	0.047

Table A1.8. Measured NH_4^+ concentration of standard NH_4^+ solutions (prepared in water) after 'extraction' in the autoclave. Concentrations are the mean of 3 or 5 measurements.

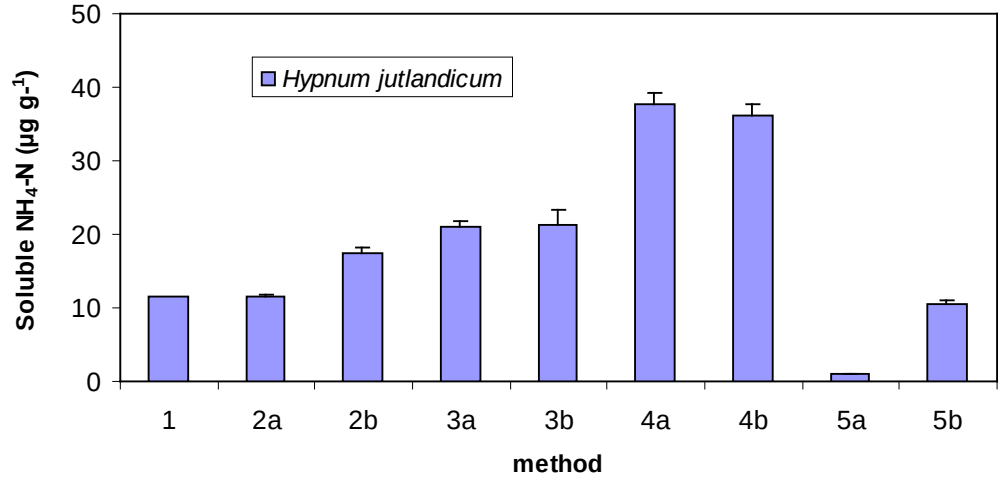
NH_4^+ concentration in standard (µg g^{-1})	**Extraction method**	**Measured NH_4^+ concentration (µg g^{-1})**	**Standard deviation**
0	Control	0.007	0.002
0	3a	0.020	0.005
0	3b	0.040	0.014
1	Control	0.985	0.004
1	3a	1.023	0.015
1	3b	1.026	0.023
10	Control	10.081	0.064
10	3a	10.116	0.137
10	3b	10.162	0.162

Table A1.9. Measured NH_4^+ concentration of standard NH_4^+ solutions (prepared in 0.1M H_2SO_4) after 'extraction' in the autoclave. Concentrations are the mean of 3 or 5 measurements.

NH_4^+ concentration in standard (µg g^{-1})	**Extraction method**	**Measured NH_4^+ concentration (µg g^{-1})**	**Standard deviation**
0	Control	0.026	0.023
0	4a	0.052	0.037
0	4b	0.052	0.035
1	Control	0.988	0.004
1	4a	1.022	0.010
1	4b	0.975	0.099
10	Control	9.327	0.725
10	4a	9.807	0.177
10	4b	9.828	0.019



a



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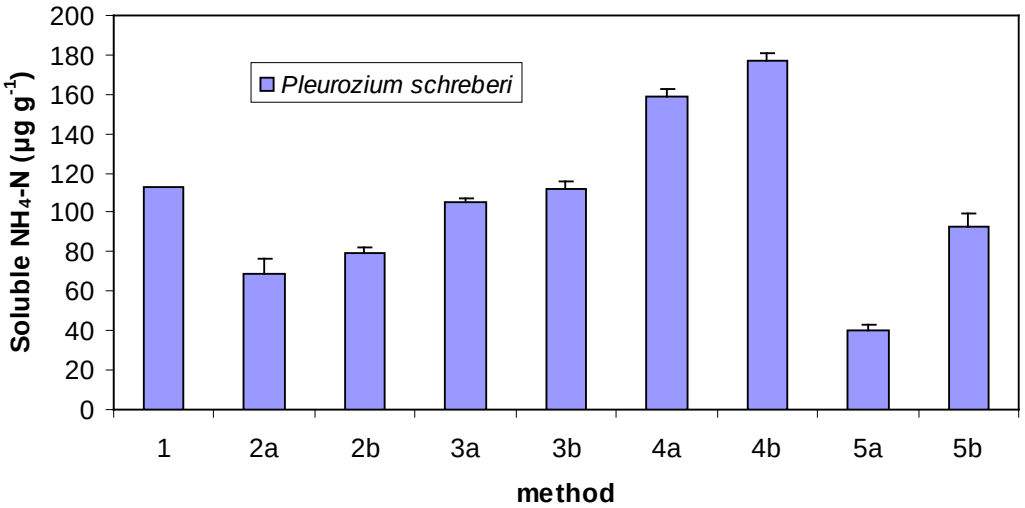


c

Figure A1.3. Soluble NH4-N concentrations in: a) *Lolium multiflorum*,, b) *Hypnum jutlandicum* collected at Whim (ambient) and c) *Pleurozium schreberi*, collected at 9m from the NH3 source at Whim, with different extraction methods. Error bars are standard deviations.

Surprisingly the 'standard' method (5a, liquid N) gave the lowest concentrations of soluble NH_4-N, although the concentration is much higher after a 24 hours 'leaking time' with the same method. Most methods with a leaking period of 24 hours gave higher concentrations then without

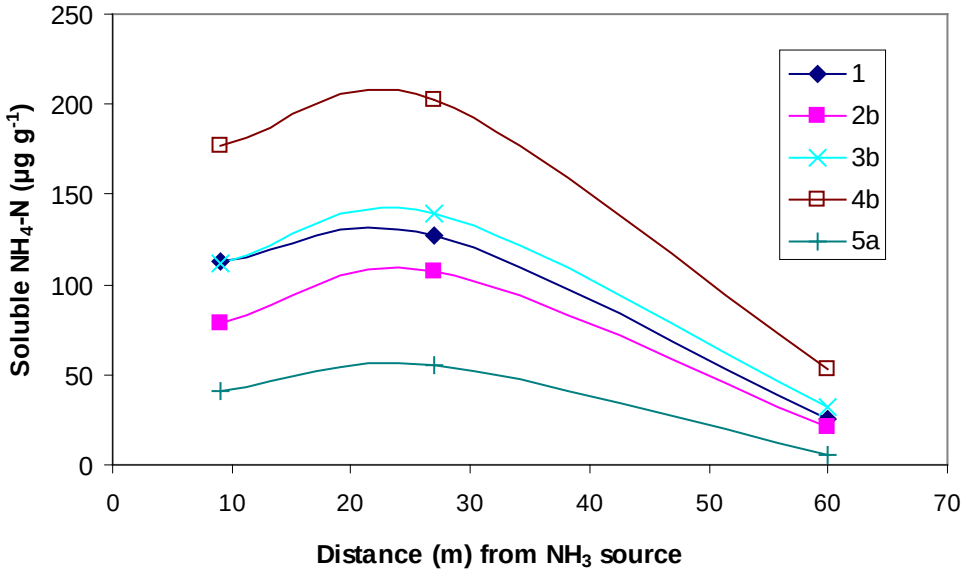
the leaking period. It appears either that the soluble N needs some time to 'leak out' of the plant material and get in the solution or that there is some biological activity in the plant material which resulted in higher NH_4-N concentration after a longer leaking time.

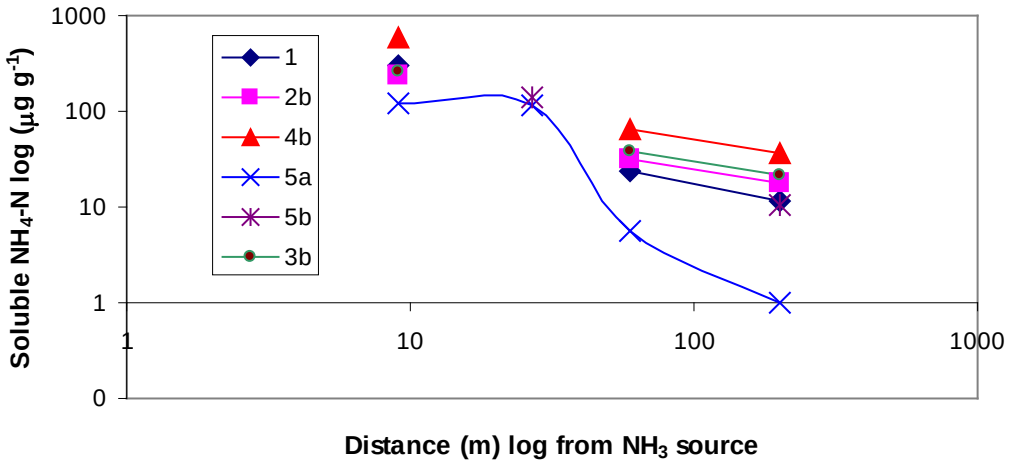
Autoclaving with water released slightly more NH_4-N than the sonic bath method but considerably less than the autoclaving with H_2SO_4, which gave the highest NH_4-N concentrations in all 3 species (*L. multiflorum, P. schreberi and H. jutlandicum*). This may be due to the acid extracting more NH_4^+, for example, as NH_4^+ is exchanged for H^+ at cation exchange sites, or due some breakdown of organic N compounds to liberate NH_4^+ dues to the acid.

Also surprising is that the most simple method (method 1, plant material in water) gave comparable NH_4-N concentrations to most of the other methods. It is not clear why method 1 gave larger values for NH_4^+ than method 2b.

b) Effects of NH_3 fumigation on NH_4^+ concentration

As the *Pleurozium schreberi* at 60 m was less exposed to NH_3 than at the other two distances (9 and 27 m from NH_3 line source) we expect lower NH_4^+concentrations in the 60m-plantmaterial. As expected the NH_4^+ concentrations were lower in *Pleurozium schreberi* sampled at 60 m than at 9 and 27m from the NH_3 line source (Figure A1.4 and Table A1.3). At all three distances along the transect, the 'standard' method (5a) using liquid nitrogen, gave the lowest NH4-N concentrations compared to the other methods. Although the *Hypnum jutlandicum* moss samples are collected from different distances from the NH_3 line source we see the same pattern: lower NH_4-N concentrations further from the line source and the 'standard' extraction method (method 5a) gave the lowest NH4-N concentrations (Figure A1.4b and Table A1.4). As with the extractions methods study method 4b consistently gave the highest NH_4^+ concentration. The NH_4^+ concentrations found for method 1 were approximately midrange at the three distances along the transect compared to the other methods.





b

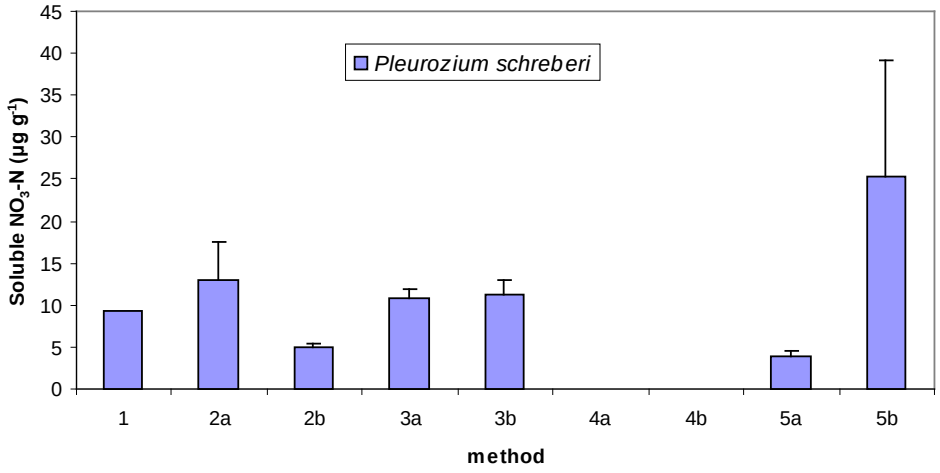
Figure A1.4. Soluble NH_4-N concentrations in *Pleurozium schreberi* (a) and *Hypnum jutlandicum* (b) extracted with different methods. The moss samples are collected at different distances from the NH_3 source at Whim Moss.

It is surprising that the highest Nf,NH_4^+ values were determined for 27 m, since the measured NH_3 concentrations at this distance (22 ug m^{-3}) of the experiment are on average smaller than at 9 m (95 ug m^{-3}). However, this effect is seen consistently between the different extraction procedures. It is also notable that the N content (% N) of the *P. schreberi* samples was highest at 27 m (1.95%, Table 2.5) and lowest at 60 m (1.46%), with an intermediate value at 9 m (1.88%).This effect may be attributed to two possible causes: a) there is a large vertical concentration profile of NH_3 immediately down wind of the source, so that the measurements of NH_3 concentrations shown here (0.1 m above the canopy, or 0.5 m above ground) are not representative of concentrations at the moss layer 0.02 m above ground. b) the accumulated deposition at 27 m may actually be larger than that at 9 m, since at the larger distance NH_3 air concentrations will have a smaller geometric standard deviation. This is to say that at 9 m the mean concentration is dominated by short periods of extremely high concentration, which are associated with low deposition velocities due to cuticular saturation. These points are a matter of interest in ongoing investigations at this experimental site.

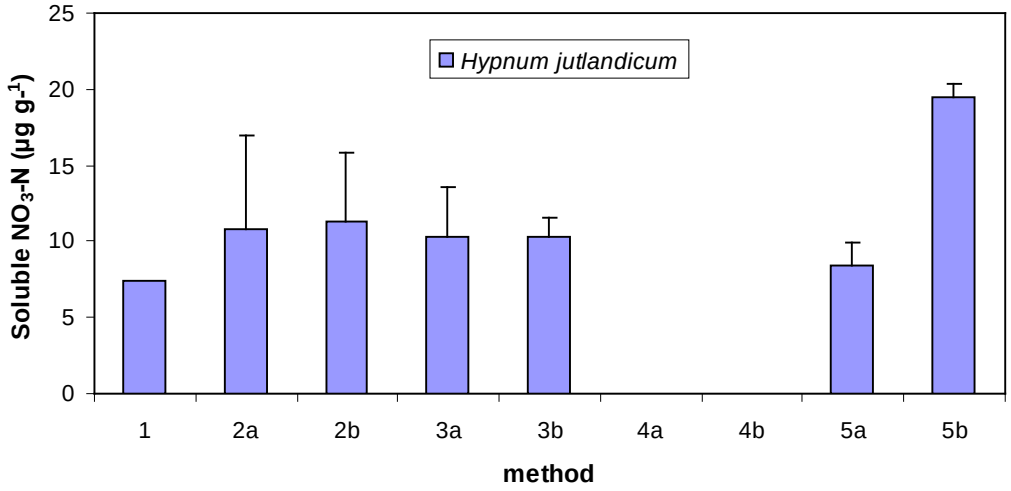
c) Soluble NO_3^-

The values of $N_{f,NO3-}$ in samples of both moss species was found to be in the range 0-14 µg g^{-1} FW which is much lower than that for $N_{f,NH4+}$, see Tables A1.3 and A1.4 and Figure A1.5 Figure A1.5 shows that there was no clear difference in NO_3^- concentration between the 5 different extraction methods tested. The concentrations in *Hypnum jutlandicum* are lower than in *Pleurozium schreberi* because *Pleurozium schreberi* was exposed to NH_3 fumigation and *Hypnum jutlandicum* not.

In the NH_3 transect study with *P. schreberi* there were also no relevant differences in NO_3^- concentrations with distance from the line source (Table A1.3). However, there were some indications that $N_{f,NO3-}$ actually increased with distance from the line source of NH_3. The scatter in this effect means that it remains a question for further investigation.



a



b

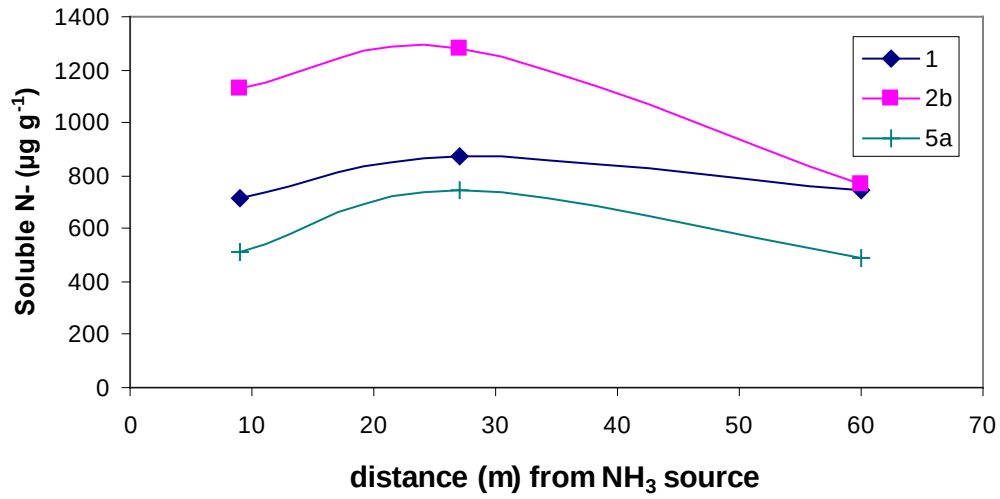
Figure A1.5. Soluble NO_3-N concentrations in: a) *Pleurozium schreberi* and b) *Hypnum jutlandicum* collected at Whim. *Hypnum jutlandicum* is collected at ambient (>200 m from line source) and *Pleurozium schreberi* is collected at 9m from the NH_3 line source at Whim. The moss samples are extracted with different extraction methods. Error bars are standard deviations.

It should be noted that although the values of $N_{f,NO3-}$ were much smaller than $N_{f,NH4+}$ in the mosses, this does not by itself prevent it being a useful indicator of plant responses. However, in practical terms, the nitrate data showed less clear responses to N supply than the ammonium. By contrast, the values of $N_{f,NO3-}$ are much larger in the grass *Lolium multiflorum* (Table A1.2) than in the two moss species (Tables A1.3 and A1.4). Overall, these differences between species are as expected, reflecting the increased preference of *Lolium* for nitrate nutrition and of the mosses for ammonium.

d) Soluble N

The measurements of soluble total N were not been completed on the extracted plant material due to ongoing technical problems with the ANTEK (Chemiluminescent Nitrogen Detector). These samples are in storage and may be analysed at a later date.

The concentrations in the samples analysed so far (Table A1.2, A1.3, A1.4 and Figure A1.6) for total soluble are much higher than the NH_4-N and/or the NO_3-N concentrations, as expected as this also includes small soluble organic nitrogen compounds such as amino acids and amines.



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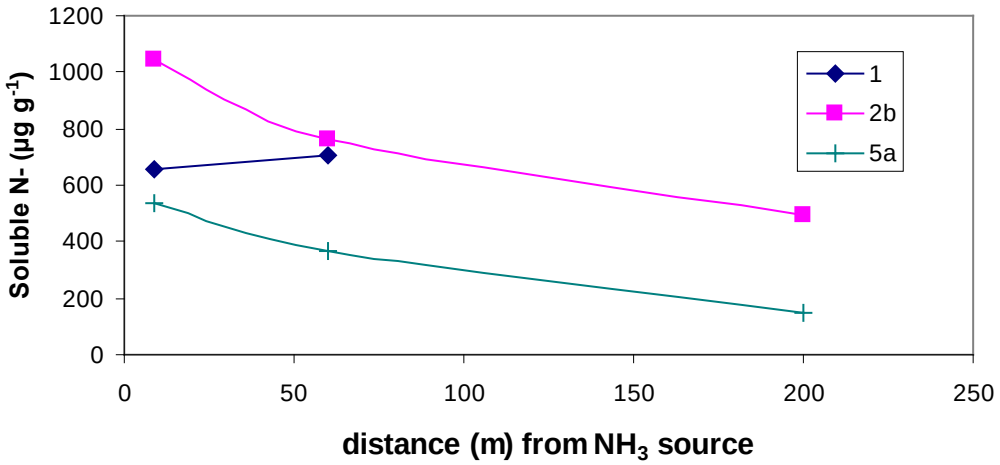

b

Figure A1.6. Soluble N concentrations in: a) *Pleurozium schreberi* and b) *Hypnum jutlandicum* collected at Whim. *Hypnum jutlandicum* is collected at ambient (>200 m from line source) and *Pleurozium schreberi* is collected at 9m from the NH_3line source at Whim. The moss samples are extracted with different extraction methods (method 1, 2b and 5a).

The tests have been carried out at laboratory temperatures of approximately 20 ^{0}C under normal daylight-night regimes. Further tests should be done tests at a range of temperatures and under different light regimes.

A1.5. Test 3: sample extracting in water: the influence of temperature, light and 'leaking time'

In test 1, the influence of time of sonication was investigated. In this test the influence of temperature, light and overall 'leaking time' on the concentration of NH_4-N in plant material was examined for material prepared according to method 1 (simple extraction in water without additional treatments).

A1.5.1. Methods

Plant material
Pleurocarpous moss samples *(Pleurozium schreberi)* were collected at Auchencorth Moss, a clean site south of Penicuik. (OS Grid reference NT220562; altitude 260 m above sea level). The plant material was cleaned and frozen as described in Section A1.2.2.

Sample preparation
1g of frozen plant material was put in a bottle and 10 ml of de-ionised water was added. There were 3 replicates of each sample. The extraction took place

a) extracting in water, 'leaking' in the lab at 20°C in the light (continuous)
b) extracting in water, 'leaking' in the lab at 20°C in the dark
c) extracting in water, 'leaking' in the cold room at 4°C in the dark

There were also samples prepared by the 'standard' method using liquid nitrogen (see test 2) and 'leaking' in the lab at 20°C in light. The 'leaking time' was respectively 0.08, 0.5, 1, 2, 4, 6, 8, 24 and 48 hours. After the extraction and leaking, the solution was filtered (PURADISC ™ 25PP disposable filter, 0.45µm Polypropylene) and stored in a -20^0C freezer until analysed for soluble NH_4^+ by AMFIA (Ammonia Flow Injection Analysis).

A1.5.2. Results

a) influence of temperature

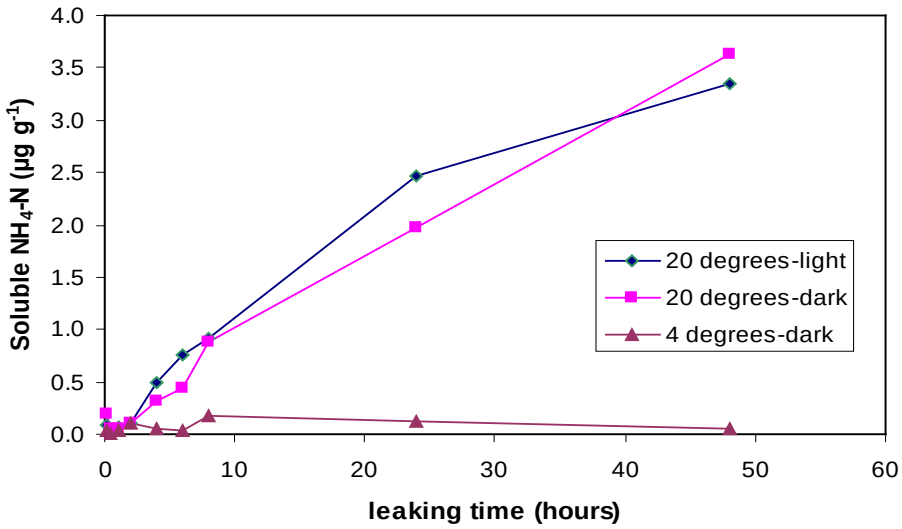

Figure A1.7. The influence of temperature during extractions on NH_4-N concentrations in *P. schreberi*. Samples were extracted at room temperature (20ºC) or in the cold room (4ºC)

At 4ºC the measured NH_4-N concentrations are much lower than the measured concentrations at 20ºC. Two processes are taking place. Firstly at 4ºC diffusion (leaking) still takes place but is slower than at 20º C and secondly at 4 ºC biological activity is virtually stopped.

b) influence of light

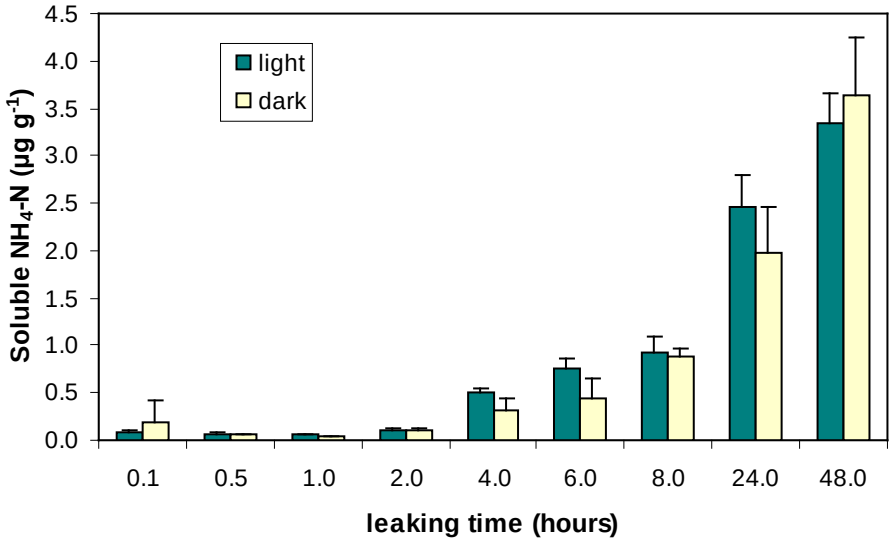

Figure A1.8. The influence of light during the extraction time on NH_4-N concentration in *P. schreberi*. Extraction took place at 20º C. Error bars are standard deviations.

Figure A1.8 shows that there is not much difference in NH_4-N concentration in *P. schreberi* samples extracted in light or dark circumstances.

c) influence of 'leaking time'

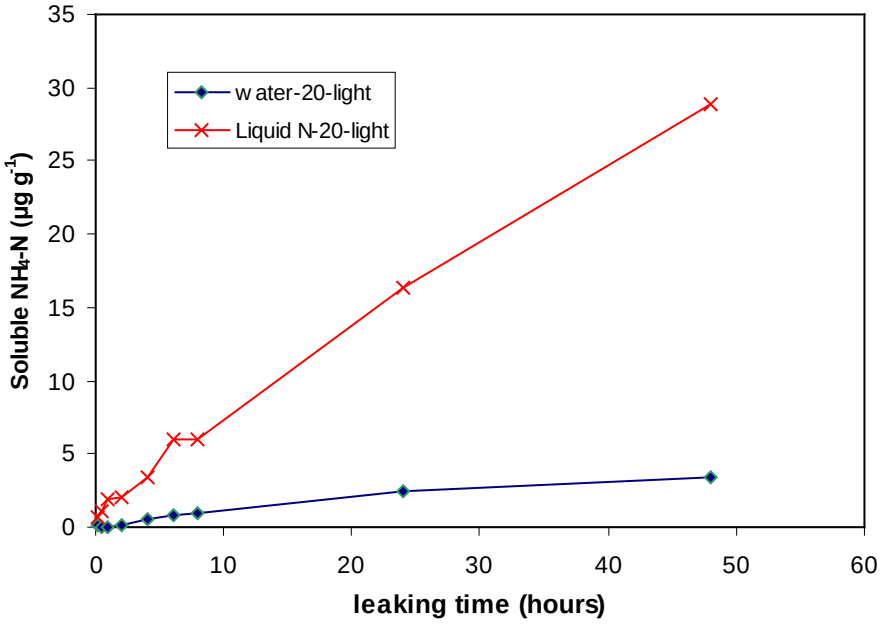

Figure A1.9. NH_4-N concentrations in *P. schreberi* after extraction in water for different times (leaking time) at 20 ^oC and after using the liquid N method.

Figure A1.9 shows that even after 48 hours leakage, the equilibriation process is not yet complete. This supports the possibility that, in addition to free soluble NH_4 leakage, biological processes are responsible for liberating NH_4^+ to the extraction solution.

It may be noted that this comparison between the water extraction (method 1) and the liquid N extraction (method 5) appears not to be consistent with that from Test 2. It should be noted that strictly method 1 is comparable with method 5b, (for a similar extraction period). Hence in Test 2, similar results were obtained between 1 and 5b, whereas Figure A1.9 shows increased values from the liquid N extract.

It is clearly preferable to measure only the free soluble NH_4 at concentrations large enough to show differences between samples. In this respect, it is notable that the present results (although they were from a clean site) showed very low values for short extraction periods (<5 µg g^{-1} FW). For that reason an extraction or leaking period of 4 hours was selected for subsequent tests.

A1.6. Test 4: Sample extracting in water: different moss species

The aim of this test was to check if the NH_4-N concentration is at the same level in different moss species.

A1.6.1. Methods

Plant material

Five species of pleurocarpous mosses, *Pleurozium schreberi*, *Scleropodium purum*, Rhytidiadelphus loreus, *Rhytidiadelphus triquetrus* and *Hylocomium splendens* were collected from the woodland around Whim where the N input is low as well as *Rhytidiadelphus squarrosus* from Bush Estate, where N inputs are higher.

Sample Preparation
1g of frozen moss material was put in a bottle and 10 ml of de-ionised water was added. Three replicated samples were collected of all species except *R. squarrosus* where only two replicates were collected. The extraction/leaking time was 4 hours for half of the samples and 18 hours for the rest. After the extraction/leaking the solution was filtered (PURADISC ™ 25PP disposable filter, 0.45µm Polypropylene) and stored in a -20^0 C freezer until analysed for soluble NH_4^+ by AMFIA (Ammonia Flow Injection Analysis).

A1.6.2. Results

The NH_4-N concentration in *R. squarrosus* was much higher than the concentrations in the other five species. (Table A1.10) This is what we expected because the N-deposition at Bush was higher than at the other site where we collected moss samples.

Table A1.10. NH_4-N concentration (NH_4-N in µg g^{-1}) in different moss species after extraction in water for 4 or 18 hours.

MOSS SPECIES	NH_4-N concentration (µg g^{-1})	
	4 hours	18 hours
P. schreberi	0.90	2.75
S. purum	1.41	4.49
R. loreus	0.87	2.50
R. triquetrus	1.54	2.76
H. splendens	1.25	7.66
R. squarrosus	20.6	111
% coeff variation (excepting *R. squarrosus)*	25%	54%

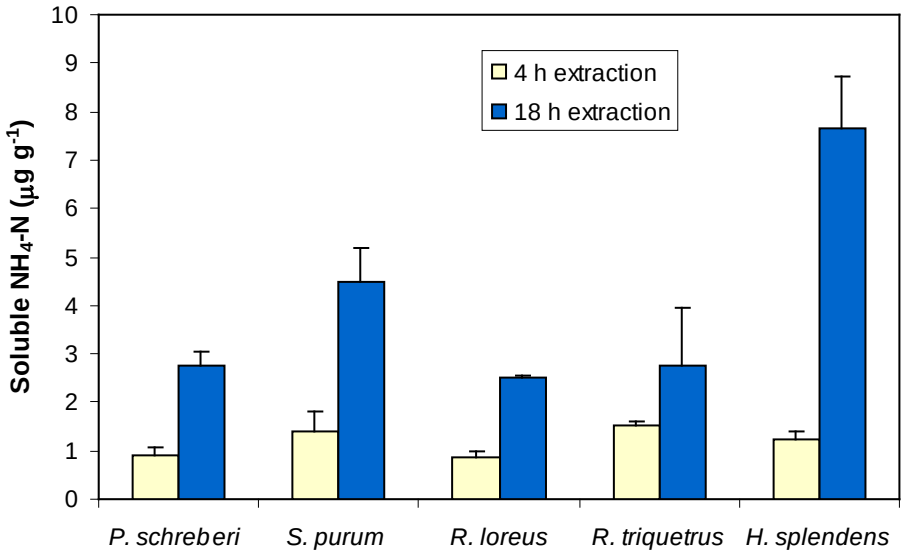

Figure A1.10. Concentration of soluble NH_4-Nin different moss species from the same clean site after 4 or 18 hours extraction in water. Error bars are standard deviations.

The NH_4-N concentrations in the five moss species from the clean site Whim are all at similar level, after an extraction time of 4 hours.

The NH_4-N concentrations after an extraction time of 18 hours are higher than after an extraction time of 4 hours and the variation between the species is also higher. For the extraction period of 4 hours the coefficient of variation was 25% while for 18 hour extraction the coefficient of variation was 54%. The larger variation for the longer extraction may reflect variations in the rate of biological processes in the samples between species, suggesting that the shorter extraction period is preferable. An extraction time of 4 hours should also be sufficient to measure sufficient NH_4-N levels in moss species, even in clean conditions.

A1.7. Discussion

Fresh plant material is used for the tested extraction methods. Although, we have not collected the plant material under very dry or very wet conditions there still will be a difference in water content of the plant material. Before weighing the sample all the samples are quickly rinsed with de-ionised water and blot dried. So the differences in water content between the samples are kept as small as possible.

Samples might be characterized by a) a free soluble part as well as by b) a biological activity part. This biological activity part is clearly affected by method of extraction, but probably also affected by the N supply. Given that b) is harder to measure (as a) and an easy method is needed, that is an argument for focusing on a).

However, then there is the practical issue to consider, that the leaking time should also be controllable so as not to make too much effect. In practice, this means the times must also be selected to match that required by standardization of laboratory TIMING, e.g. if keeping all samples to extract for 0.5 hour is impossible and then longer is needed.

When determining the leaking period it is important to avoid leakage of products of biological processes while obtaining concentrations of free soluble NH_4-N large enough to measure differences between samples. An extraction/leakage period of 4 hours in de-ionised water at 20’C (without any additional extraction procedures) satisfied these requirements, while providing a method that is simple and easily standardized.

A1.8. Conclusions

- The results of the extraction methods study show that extraction in de-ionised water for 24 hours gave NH_4^+ concentration similar to these of the more labour intensive methods. These results were consistent in all the 3 species (*L. multiflorum*, *P. schreberi* and *H.jutlandicum*) tested.

- Measurement of NH_4^+concentration in the moss plant tissue is considered a better chemical bio-indicator than soluble NO_3^- concentration, since the latter has extremely low concentrations.

- After a shorter extraction time in water of 4 hours, the levels of NH_4-N are high enough to be measured, while the measurement of effects of biological activity in the stored samples are kept small although there is some activity in this period.

- There is little influence of light during the extraction period (in water).

- Extraction in water for 4 hours was found to be the quickest, simplest and cheapest of the methods tested and is therefore used as the chemical bio-indicator method in both the Intensive and UK scale fieldwork.

- The sample preparation protocol for the extraction method used to measure free soluble NH_4 in moss material is:

    - Weigh 1 g of frozen moss material and put in small bottle
    - Add 10 ml de-ionised water
    - shake and leave to extract for 4 hours at lab temperature (20ºC)
    - shake again
    - filter the solution
    - store the filtered solution in freezer at -20ºC until analysing.

- The wide variation in measured ammonium, nitrate and total soluble nitrogen in leaf tissues according to extraction procedure demonstrates the importance of maintaining a standard methodology for these indicators. The variation is due to the different extent of diffusive extraction and biological activity in the processed samples according to methodology used.

A1.9. References

The references for this appendix are included in the main reference list.

Appendix II: Evaluating *lolium multiflorum* as a standardised grass bioindicator for gaseous ammonia

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A2.1. Introduction

One of the unknown parameters in the Phase I field study (Sutton *et al.* 2004a) using the standardised grass (*Lolium perenne*) transplants was the effect of the NH_3 concentration / N deposition on plant roots systems. In this field study, only the above ground biomass of the standardised grasses was measured. The extraction/cleaning of the root systems is labour intensive, especially when using a soil based mixture, and was out-with the scope of the Phase I study. In this current phase of the project, a pilot study was carried out using an alternative growing medium to determine if it was feasible to measure below ground biomass and nutrient status in a large scale study. Sommer and Jenson (1991) had found that much of the additional N deposition was to the roots in standardised Rye grass.

Root sample preparation i.e. root washing to remove the growing medium is the most labour intensive and time consuming part of the destructive harvest of root systems. To reduce the labour input on root cleaning, an inert, organic clay substitute (Agsorb) was used. As this was a short-term pilot study a fast growing species was required. *Lolium multiflorum* was selected, as this was the preferred standardised grass species used in the EuroBionet Study (EuroBionet 2003). The standardised grass plants were exposed to varying concentrations of ammonia at the CEH Edinburgh field release facility at Whim Moss (Leith *et al*. 2004) during May-June 2003.

A2.2. Methods

A2.2.1. Plant material

Lolium multiflorum seed (supplied by Herbiseed.Wokingham, UK) was sown at a rate of 0.8 g per pot on 15 April 2003 into 1.1 litre square pots filled with 18/40 grade Agsorb. The Agsorb was thoroughly wetted prior to sowing. The pots were then placed in an unheated glasshouse at CEH Edinburgh (Figure A2.1). The grasses germinated within 3-6 days and were left in the glasshouse until 28 April, when they were hardened-off in a sheltered location outside.

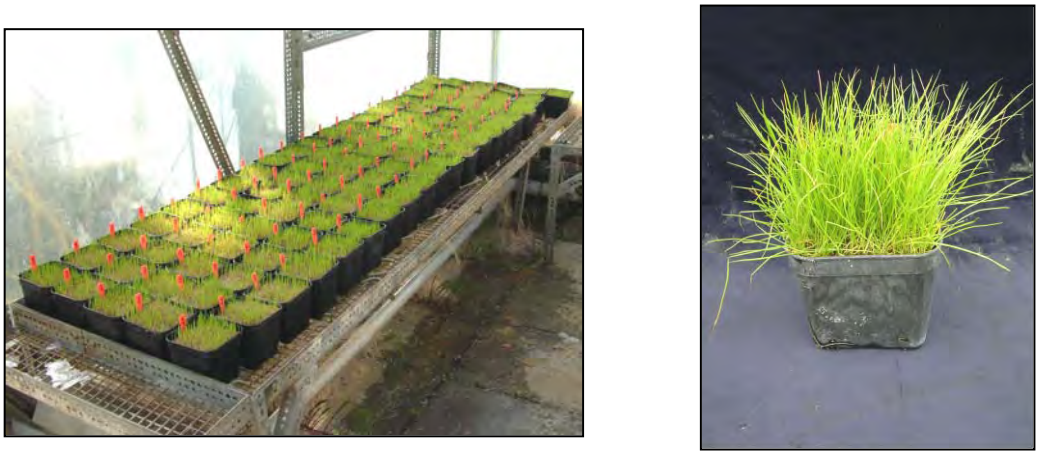

Figure A2.1. a) *Lolium multiflorum* grown from seed in a glasshouse at CEH Edinburgh. b) A single pot of *L. multiflorum* after one month of exposure to mean NH_3 concentration of ~40 μg m^{-3}.

The grasses in all the individual experimental pots were cut back to 2-3 cm above the pot rim prior to exposure at Whim Moss on 30 April 2003. This was done to prevent the grasses becoming too elongated and also to encourage growth during the exposure period. They were also fertilised, with each pot receiving 50 ml of 0.05 M KNO_3 solution (0.035 g N per pot)

A2.2.2. Agsorb

Agsorb (trade name Terra-green) is used commercially as an inorganic, inert soil conditioner, being an absorbent granular calcined attapulgite clay. Agsorb was used as a soil substitute in the 2003 *L. multiflorum* study to allow root biomass and root N content to be determined, as it is easier to remove from root structures than conventional soil mixtures.

Agsorb supplied by Oil-dri (UK) Ltd, Wisbech, Cambridgeshire, UK; has a high cation exchange capacity, which allows it to absorb nutrients and make them available to the roots. It has a low N content of approximately 1 mg g^{-1}. Agsorb calcined structure allows it to continue supplying the root system with water, nutrients and oxygen indefinitely. It is used in arbuscular mycorrihizal research as it provides a sterile medium with no organic matter.

A2.2.3. Pre-treatment harvest

On the 30 April 2003, 10 individual plants were cut and the foliage collected for pre-treatment determination of total foliar N content (% dry weight) biomass and hence total N inventory.

A2.2.4. Experimental procedure (Whim Moss Experimental facility)

Using the existing NH_3 transect at Whim Moss experimental facility (Leith *et al.* 2004), a single tray each containing six *L. multiflorum* plants was placed at distances of 2 m, 4 m, 8 m, 16 m, 32 m and 60 m away from the ammonia release point (10 m line source) and also at ambient background on 1 May 2003 (Figure A2.2). The tray/wicking system used is shown in Figure A2.2. The trays were positioned on the central boardwalk at a height of 0.5 m above the ground (Figure A2.2). The plants were initially to be exposed for one month, however, due to rabbit damage to individual plants at two distances the plants were exposed for two months with all the above ground vegetation being cut back to a uniformed height after 2 weeks exposure.

A2.2.5. NH_3 concentration monitoring

The ambient NH_3 concentrations were monitored at the seven distances away from the NH_3 point source by using passive NH_3 samplers (either diffusion tubes or ALPHA samplers. See Section 2 for a description of NH_3 monitoring methodology).

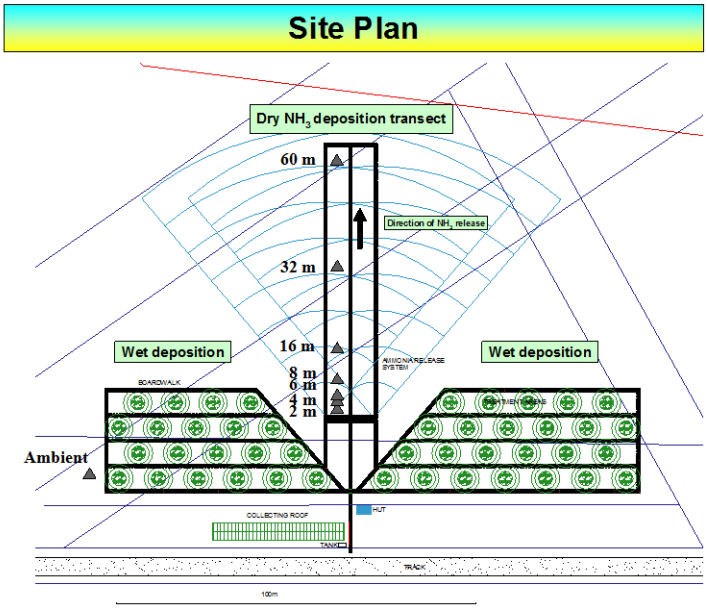

Figure A2.2. Schematic diagram of the locations of the *L. multiflorum* plants along the NH_3 transect at Whim Moss.

A2.2.6. Destructive harvest

After exposure for 61 days all pots were returned to CEH Edinburgh on 30 June 2003 for destructive harvest. To provide sufficient plant material for total N content three duplicate samples were taken by bulking two replicate pots (2 pots x 3 replicates). Using disposable gloves tillers were cut at 2 -3 mm above the level of the pot.

A2.2.7. *L. multiflorum* tiller and root biomass and tissue N content

The tillers were washed in de-ionised water to remove any surface dry N deposition, oven dried at 70 ^{0}C for 3 days then dry weighed. The dried samples were then ground using a hammer mill (sieve size 0.8 mm) and digested and analysed by the CNS method (Grimshaw *et al.* 1989). The roots systems were hand washed to remove all the Agsorb particles, oven dried and weighed for total biomass and then analysed for total N content using the same procedure as for the above ground material.

A2.3. Results

The NH_3 concentrations (at a height of 0.5 m above the vegetation along the 60 m NH_3 transect) were similar for both months of the study (May and June 2003). The NH_3 concentrations ranged from 200 μg m^{-3} at 2 m from the NH_3 line source to 0.5 μg m^{-3} at ambient background in June 2003 (Figure A2.3).

There is a exponential decrease in NH_3 concentration with distance from the line source. The differences in NH_3 concentrations between May and June at Whim Moss is due to NH_3 release being dependent on prevailing weather conditions i.e. when wind is in the sector 180-215^0 and the windspeed is greater than 2.5 m s^{-1}.

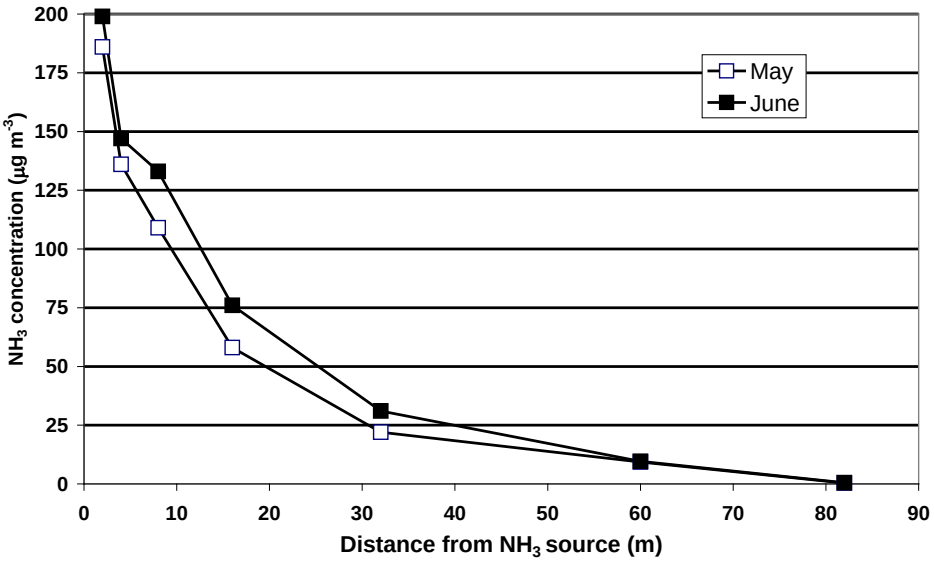

Figure A2.3. Mean NH_3 concentrations at Whim Moss for May and June 2003.

A2.3.1. Above and below ground biomass

The above-ground and below ground biomass of *L. multiflorum* decreased linearly ($R^2 = 0.835$ and $R^2=0.589$ respectively) with the log of distance from the NH_3 line source (Figure A2.4). There was a greater root biomass compared to the above ground biomass at each of the distances along NH_3 gradient.

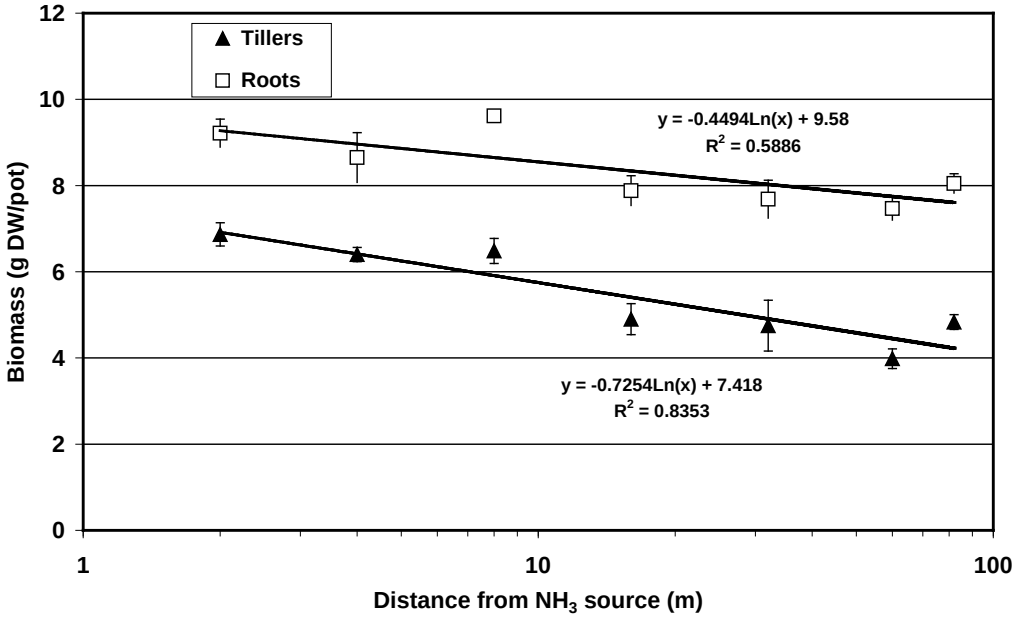

Figure A2.4. *Lolium multiflorum* above and below biomass ± 1 SE with increasing distance from the NH_3 line source at Whim Moss.

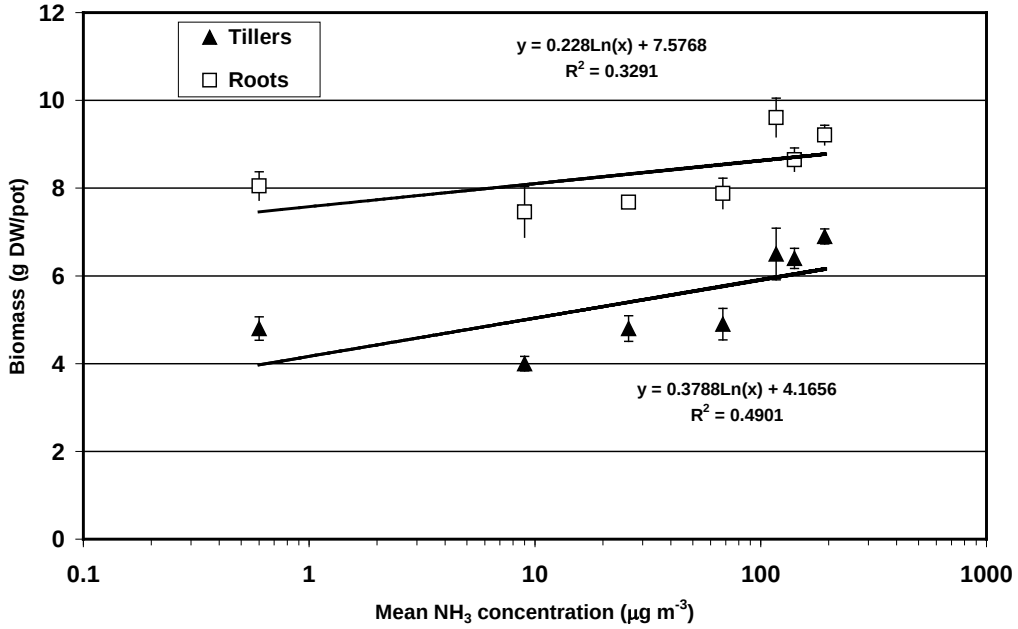

Figure A2.5. *Lolium multiflorum* above and below biomass ± 1 SE with increasing NH_3 concentration at Whim Moss.

Measurements of above ground biomass showed a linear relationship with the log of NH_3 concentration for *L. multiflorum* after 2 months of NH_3 treatment. The above ground biomass increased with increasing NH_3 concentration from 4.8 g dry weight/pot in the ambient treatment (0.5 µg m^{-3}) to 7 g dry weight/pot at 200 µg m^{-3} (Figure A2.5). A similar increase was found in the root biomass with increasing NH_3 concentration. The above ground biomass accounted for 63% of the overall response whereas the root biomass was lower at 37%.

A2.3.2. Tissue N content

Both the above and below ground percentage N content decreased linearly with distance from the NH_3 source (Figure A2.6). The linear relationships of both shoot and root growth with distance indicate that there is N uptake even for this short time period (2 months) although the effect of the increased NH_3 concentration is larger in the above ground vegetation (76% of the overall response). There were also strongly linear relationships between biomass and NH_3 concentration for both above and below ground biomass accumulation (Figure A2.7). The regression lines for the above ground biomass are again steeper for NH_3 concentration than for the roots, accounting for 73% of the overall response to *ln* NH_3 concentration

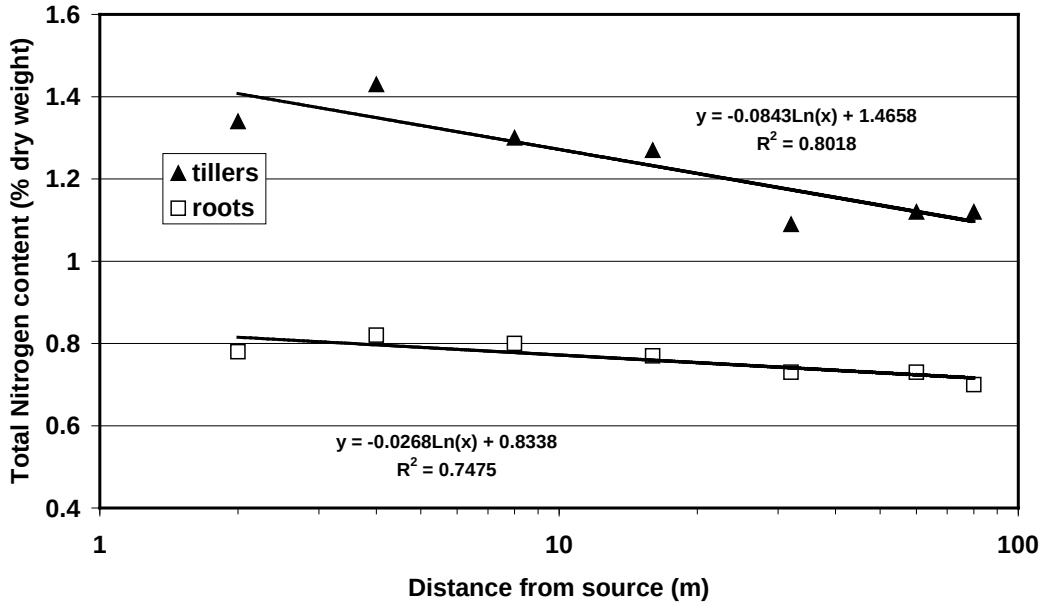

Figure A2.6. *Lolium multiflorum* above and below total N content with increasing distance from the NH_3 point source at Whim Moss.

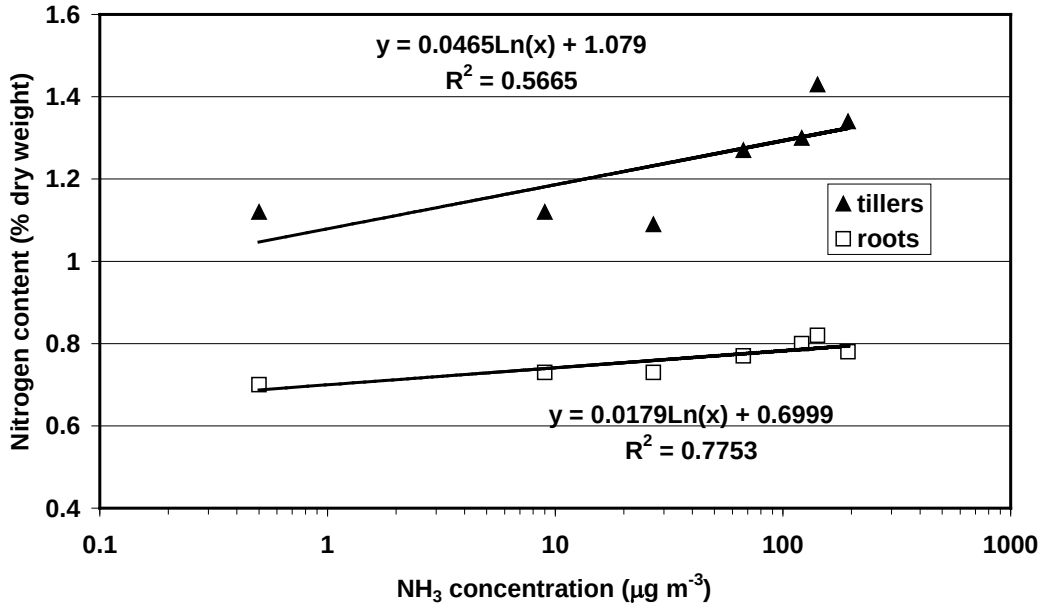

Figure A2.7. *Lolium multiflorum* above and below total N content with increasing ambient NH_3 concentrations at Whim Moss.

A2.3.3. Total above and below ground inventory

The N content expressed as the total above ground N inventory shows that the N levels decreased with distance from 70 mg N per pot close to the source to 52 mg per pot at 80 m from the source. There is a stronger relationship between N and above ground vegetation i.e.R^2 = 0.7435 compared to the below ground, R^2=0.5665. (Figure A2.8). The measure of total plant inventory of N combines the larger response shown for above ground plant material compared with below

ground for both biomass and % N. As a consequence, for the total plant N inventory, 70% of the total response (according to *ln* distance) occurs in the above ground material, with only 30% in the roots.

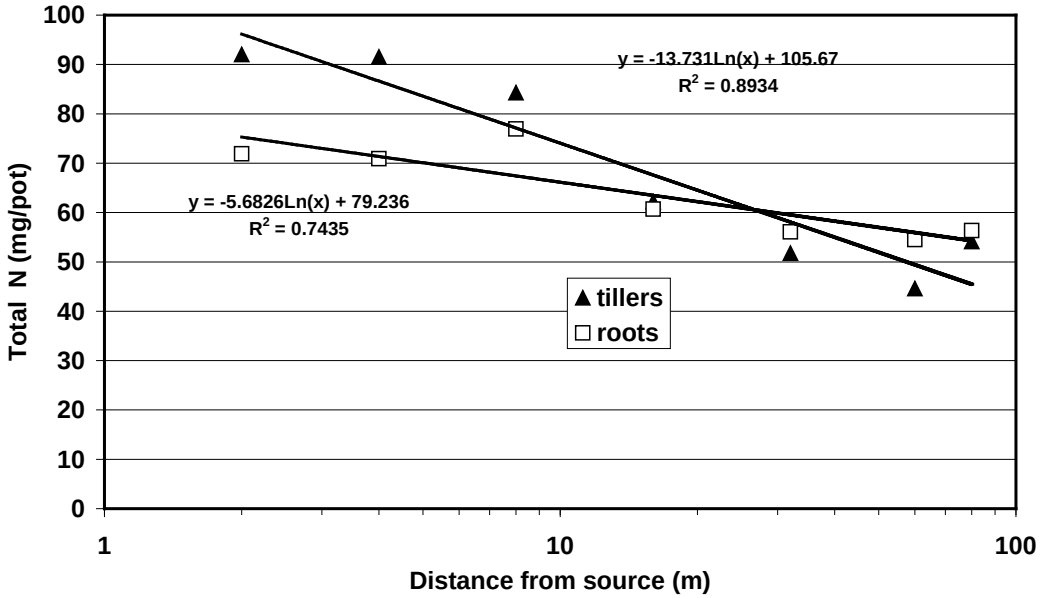

Figure A2.8. The relationship between total above and below ground N per pot (mg N) for *Lolium multiflorum* and the distance from the NH_3 point source at Whim Moss.

A trend of increasing N expressed as mg/pot for both above and below ground vegetation with NH_3 concentration was also found (Figure A2.9). The N inventory log linear relationship was stronger than that for distance from the NH_3 source with R^2=0.918 for tillers and R^2 = 0.793. As with the relationship to distance from NH_3 source, the response of the plant N inventory to log NH_3 concentration was dominated by the tillers (70%) compared with the roots (30%).

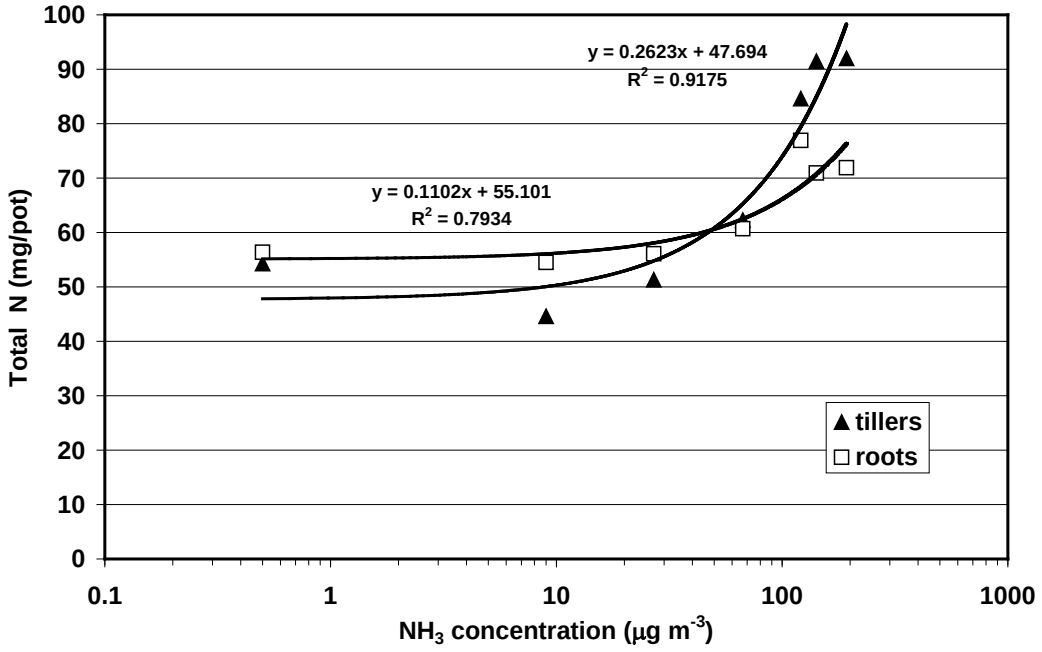

Figure A2.9. The relationship between total above and below ground N per pot (mg N) for *Lolium multiflorum* and the log NH_3 concentrations at Whim Moss.

A2.4. Discussion

This pilot study, which calculated both above and below ground biomass and measured the N content is novel as most studies do not attempt to determine the below ground parameters.

The results show that *L. multiflorum* is a robust species to use as a standardised grass bioindicator as there was a clear biomass response and an increase in N content along the NH_3 gradient.

The results show a differential response to enhanced N concentrations between the tillers and the roots. In general, increased N concentrations stimulated above ground biomass and N content. The N concentration in *L. perenne* measured in the field inter-comparison (Sutton *et al.* 2004a) was higher than that for *L. multiflorum* in the current study. Leith *et al*. (1999) found that root growth in *Eriophorum vaginatum*, *Erica cinerea* and *Vaccinium vitis-idaea* did not respond to N additions. The results show that the above ground foliage responded to additional gaseous NH_3 inputs over a short 2month period indicating that *Lolium multiflorum* is a potential bioindicator for use close to an N point source. The use of Agsorb speeded up the root washing process considerably but it was still very labour intensive taking approximately half an hour plus per root system. The scale of the main standardised grass study to be conducted in 2004 at the 5 intensive sites would make it impossible to determine the root biomass and nutrient parameters.

Therefore, although it is interesting scientifically, the impacts on roots are not considered a potential bioindicator. This is underlined by the results here, which show that the overall response to additional NH_3 is dominated by the plant tillers for biomass (76%), % N (73%) and total N inventory (71%).

A2.5. Conclusions

- *Lolium multiflorum* was found to be a suitable species for use as a standardised grass bioindicator with a defined NH_3 point source under experimental field conditions.
- The *Lolium multiflorum* above ground biomass increased significantly, whereas there was a much smaller response in the roots biomass with increasing NH_3 concentrations.
- There were strong linear increases in both above and below ground tissue N content (% dry weight) with log NH_3 concentration, although the above ground appears to be more sensitive to the enhanced NH_3 concentrations.
- Agsorb worked successfully as a growing medium for *Lolium multiflorum.*
- Although the extraction/cleaning time of the roots was speeded up using Agsorb compared to soil/peat based composts it is still very time consuming.
- The use of roots as an N bioindicator is considered not suitable for large scale-studies.

Appendix III: Lichen sampling Protocols

P.A. Wolseley

Department of Botany, The Natural History Museum, London

A3.1. Background

Lichens on corticolous substrates (trees) have been used as indicators of changes in pollution levels for many years across Europe. This project addressed the need for a standardized method of collecting lichen data on trees that can be used in a range of conditions across the British Isles. In the Netherlands, van Herk (1999) used lichens on trunks of wayside oak trees combined with a weighted scoring system to define indicators of acidification and nitrification. The trialling of this system in Britain demonstrated the need for regional definition of indicator species (Wolseley and James 2002b). In addition use of data from twigs demonstrated the rapid response of lichens on younger substrates (Wolseley and Pryor 1999, Wolseley and James 2002b).

Following a meeting of specialists from across Europe a standardized method of sampling lichens on tree trunks was agreed (Asta *et al*. 2002), using frequency of lichens on four cardinal points between 1-1.50 m. The use of frequency data allowed an investigation of changes occurring in lichen communities with time and conditions. When this was also applied to twigs the data showed that a simpler recording system using macrolichens only provided a good correlation with ammonia deposition (Sutton *et al*. 2004a). Although twig age was originally estimated using girdle scars and scored by year (Wolseley and Pryor 1999), it proved more practical to estimate frequency of macrolichens by dividing each twig into three zones (Wolseley and James 2002b).

Bark pH has been shown to have a considerable effect on lichen communities, and as tree species vary considerably in bark pH previous surveys have attempted to use the same tree species. This restriction was not possible in sites scattered across a range of environmental conditions with a range of available tree species. In order to investigate the impacts of substrate, bark pH and ammonia all tree species were recorded and bark samples from trunks and twigs collected. Estimation of bark pH in the lab allowed us to investigate the relationship between bark pH, ammonia deposition and lichen communities.

Lichen Sampling Protocol

trunks and twigs of selected trees

Site selection.

1. Select a sampling site with appropriate tree species (see below) as close to the air monitoring station as possible. Note distance from the air monitoring sampling station and conditions on form.

2. At the site locate 3-5 standard trees **of the same species** with girth >40 cms (where possible use acid-barked trees).

3. Sample **± straight trees with well exposed trunks** (e.g. field or wood edges, parkland) **and twigs** (avoid shaded sites e.g. interior of woods). The trunks and twigs lichens will be recorded separately.

Bark pH	**Tree species**
Low (acid bark)	Oak, Birch, Alder, Sweet Chestnut, Rowan, Hawthorn, Hornbeam, Pine, Spruce
Medium	Hazel, Ash, Sycamore, Willow, Lime
High (base-rich bark)	Elm, Field Maple

*Beech trees not suitable due to heavy shading of lower branches in summer.

METHOD

Equipment:

You will need a **x10 hand lens*, twig key and 3 recording forms for each site.**

Plastic ladder quadrat 10 x 50 cms internally, knife*, compass*, measuring tape*, pencil and chlorine*+ (see lichen key) for testing lichen species.

+ chlorine is obtainable as the cheapest household bleach (more expensive bleach has other chemicals added which may produce a yellow colour). It must be used fresh (still smells strongly of chlorine) to obtain red to pink colour reaction.

Paper collecting bags for lichen specimens and bark samples.

Enter details of each sampling site on the site recording form including adjacent land use and distance from the air monitoring station.

LICHENS ON TWIGS KEY (FSC FOLD-OUT KEY) PLUS TWO A5 SHEETS.

***EQUIPMENT NOT INCLUDED IN SAMPLING PACK**

The hand lens, knife, compass chlorine and a measuring tape have not been included

in the pack on the assumption that local agency offices would hold these.

RECORDING METHOD - FOR EACH TREE:

- Record tree species and girth at 1.50 m above ground of each tree sampled
- Place plastic ladder quadrat with five 10 cms squares with top at 1.50 m above ground level on N aspect of trunk, using drawing pins or strong pins to hold it in place (Figure A3.1).
- Familiarise yourself with the lichens in each sample using the illustrated key.
- Record presence of species of foliose and/or fruticose lichens in each 10 cm square on form (see page 295).
- Record presence of crustose lichens, bryophytes, algae and bare bark in each 10 cm square (not necessary to record these to species level, just note presence).
- Repeat on all compass points (N, E, S, W) so that presence in 20 squares is recorded for each component on the trunk.
- Add up the mean number of all NI and AI species for both trunks and twigs and record on form (Figure A3.5)

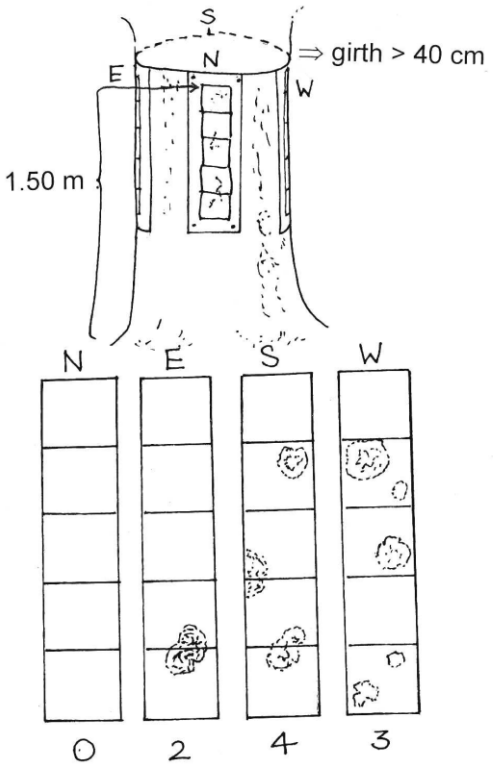

Figure A3.1. Schematic diagram showing sampling positions and scoring using plastic quadrat ladder.

Bark pH - trunks.

- In the vicinity of the sampled quadrats select bark without cryptogam cover and with smooth area >1 cm diameter avoiding rough surfaces and crevices (to allow pH determination using a flat–tip electrode 1 cm diameter).
- Using a fixed blade knife collect 2 bark samples between 2-3 cms diameter from each tree (Figure A3.3).
- Air dry bark samples in labelled **paper** bags for subsequent pH determination.

TWIGS

WITHIN THE SELECTED SITE, ON THE SAME TREE SPECIES SAMPLED FOR EPIPHYTES ON TRUNKS, SELECT EXPOSED AND ACCESSIBLE TWIGS (NOT NECESSARILY THE SAME TREES AS SAMPLED FOR TRUNKS). AVOID SHADED TWIGS.

- Record tree species and aspect of twigs, and adjacent land use on site form (Figures A3.4).
- Familiarise yourself with lichens present on the twigs using the twig key, noting difference between presence of superficial and immersed species (*Arthonia* and *Arthopyrenia* species) on younger bark.
- Select 5–10 accessible twigs at each station. If necessary anchor them temporarily within reach using rope and a weight (rucksac or rock!).
- Identify fruticose and foliose lichens to species where possible (species of *Usnea* and *Hypogymnia* are difficult to identify when young and may be recorded as their genus (species in these genera are all acidophytes)).
- Record in 3 measured zones on each main twig discounting side branches and starting from the youngest woody growth: 0-20 cms (excluding new growth), 21-50 cms and 51-200 cms (Figure A3.2).
- Record presence of fruticose and foliose lichens at species level in each zone. Also record presence of crustose and immersed lichens, bryophytes and green algae in each zone (not necessary to record these to species level, just note presence) (pages 295-296).
- Unidentified fruticose and foliose specimens can be collected, labelled, dried and stored in **paper** envelopes or bags (not polythene) and sent together with bark specimens for pH (Figure A3.3).

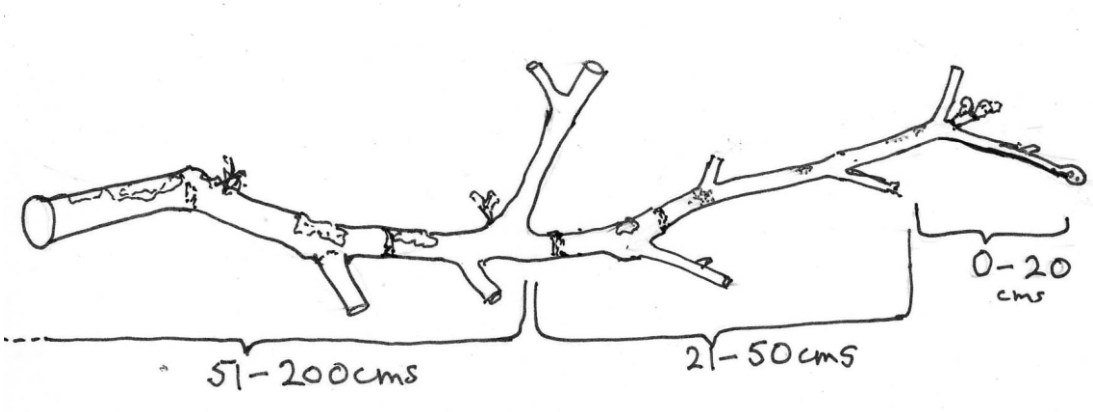

Figure A3.2. Diagram of twig showing the three measured zones starting from the youngest woody growth.

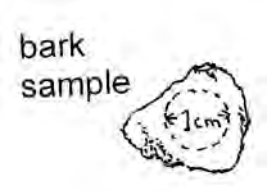

Figure A3.3. Schematic diagram of bark sample for bark pH determination.

Bark pH - twigs.

- Collect ± straight portions of 5 twigs at least 10 cm long and c. 5-7mm in diameter (straight section should be c. 6 cm long and without branches so that it fits in a test tube).
- Air dry and store in **paper** bags for pH determination.

The field sampling data sheets are shown below (pages 295-296). These were sent out to the UK conservation agencies field staffs that were carrying out the sampling for the UK extensive study.

Once completed the sheets along with the bark and twig samples were returned to CEH and then sent to Pat Wolseley at the Natural History Museum, London for analysis.

LICHENS ON TRUNKS AND TWIGS recording form

1: site

Recorder:	Date:

Site name:

Location (parish/town/village/ county):

Grid reference (map co-ordinates):	Altitude (in metres):

Site description (describe the site environment e.g. a hedgerow or boundary trees, garden, and if shaded or exposed):

Adjacent conservation site, name, status and distance from NH_3 monitoring station

Land Management (tick land management type immediately adjacent to the station):

	✓		✓
Arable fields		Houses	
Pasture		Road	
Farmyard		Woods	
Churchyard		Other (please specify)	

Figure A3.4. Site data sheet

RESULTS

Distinguish foliose and fruticose nitrophytes and acidophytes in your form from the following list:

Nitrophytes (NI)	**Acidophytes (AI)**
Diploicia canescens (on trunks)	*Evernia prunastri*
Hyperphyscia adglutinata (on trunks)	*Hypogymnia spp.*
Parmelina spp.	*Flavoparmelia caperata*
Phaeophyscia orbicularis (on trunks)	*Parmelia saxatilis*
Physcia adscendens or P. tenella	*Parmeliosis ambigua or Cladonia spp.* (on trunks)
Physconia spp.	*Platismatia glauca* or *Cetraria chlorophylla*
Xanthoria parietina	*Pseudevernia furfuracea*
Xanthoria polycarpa	*Usnea spp.*

Add up mean number (last column) of all NI and AI species at each station to give you the nitrophyte and acidophyte indices.

	Tree species	**AI**	**NI**
Trunks			
Twigs			

Figure A3.5. Recording form for AI and NI species from the trunks and twigs**.**

LICHENS ON TRUNKS RECORDING FORM

SITE NAME…… Station ref no:…… Tree species ……..

	T1				T 2				T3				T4				T5					
LICHENS	N	E	S	W	N	E	S	W	N	E	S	W	N	E	S	W	N	E	S	W	*Total Value*	*Mean* (= divided by no. of samples)
Crustose species																						
Immersed lichens																						
Bryophytes																						
Green algae																						
Bare bark																						
TOTAL number of lichen species on each trunk	**T1**				**T2**				**T3**				**T4**				**T5**					

LICHENS ON TWIGS RECORDING FORM

SITE NAME…… Station ref no:…….

Tree species ……..

	Twig 1			Twig 2			Twig 3			Twig 4			Twig 5			Twig 6			Twig 7			Twig 8			Twig 9			Twig 10				
LICHEN SPECIES																															*Total Value*	*Mean* (= divided by no. of samples)
Crustose lichens																																
Immersed lichens																																
Bryophytes																																
Green algae																																
TOTAL lichen species per twig																																