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Opportunities for Using DNA in Terrestrial Monitoring

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This project was led and funded by the Defra DNA Centre of Excellence in 2019/20, which aims to transform Defra group science to support policy and delivery by sharing and demonstrating the wider potential of existing methods; enabling the rapid development and trialling of new DNA based method applications; providing a focal point for leadership in Defra, UK and across OGDs and building shared capability including skills and facilities.

DNA | COE

Summary

- DNA-based methods could improve the breadth and efficiency of the monitoring carried out in terrestrial systems by environmental public bodies.

- The project aimed to understand the requirements and constraints in current monitoring (particularly those involved with the Defra DNA Centre of Excellence), and so identify where the greatest need for improvement lies. This can help to guide future development of DNA-based methods.

- Information was gathered by individual interviews with one or more specialists in each organisation and by inviting comment through e-mail. Key questions covered **(i)** the species/assemblages of interest, **(ii)** the main driver(s) for the monitoring, **(iii)** the magnitude of current and required sampling, **(iv)** the main constraints and problems in current approaches, and **(v)** an overall assessment of the effectiveness of the current approach.

- 19 responses were received (including 13 interviews), covering Natural England, Environment Agency, Defra Plant Health, Forest Research, Forestry England, SNH and NRW[1].

- Five key areas of shared interest across multiple organisations were[2]:

  I. **Assemblage-level invertebrate monitoring methods** that help alleviate constraints on the availability of taxonomic expertise and can be applied across multiple habitats and purposes (NE, Environment Agency, Forest Research, Forestry England, Defra Plant Health, SNH, NRW).
  II. **Assemblage-level fungal monitoring methods** that address the limitations in aboveground survey methods and the problems posed by taxonomic revisions (NE, SNH, NRW, Forestry England).
  III. **Methods for monitoring soil biodiversity** covering a range of purposes – e.g. national monitoring, informing payments to farmers, understanding pollution impacts, woodland management (NE, Environment Agency, Forest Research, Forestry England, Defra, SNH).
  IV. **Methods for surveying mammals** that reduce the need for multiple visits and can reliably detect several species. Specific interest in using eDNA from waterbodies, particularly if this can also detect terrestrial mammals (NE, SNH).
  V. **General metrics that provide information on the effects of pressures and interventions, natural capital *etc.*** (all organisations).

- These topics seem amenable to DNA-based methods, but require varying degrees of additional work to implement operationally – e.g. cross-validation with conventional surveys to ensure comparability, agreement on protocols, incorporation into regulatory frameworks, *etc*. Incomplete reference libraries also pose problems for several applications, and so the priority topics identified here could help inform strategies for progressively filling these gaps.

[1] NB: Additional organisations and individuals were also invited to provide input but a response was not received.
[2] Lack of a response on a particular theme does not necessarily reflect a lack of interest within an organisation.

- Potential next steps for topics (I-IV) are subject to interest from key individuals and organisations, but could be:

    1. Targeted workshops/telecons to bring together end users and academics. These would identify the current barriers to operational use and a series of activities needed to address these barriers.
    2. The activities would effectively form a consensus workplan, which could allow more strategic investment in key projects, identify areas for cross-organisation collaboration, help researchers and end-users co-develop relevant projects (e.g. as part of studentships), and provide a stronger case for funding key activities.

- Topic V could be partly addressed in the above discussions but should also link with the related Defra DNA Centre of Excellence project on developing new metrics.

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List of Abbreviations

CoE	Centre of Excellence
Defra	Department for Environment, Food and Rural Affairs
EA	Environment Agency
FE	Forestry England
FR	Forest Research
NE	Natural England
NRW	Natural Resources Wales
SNH	Scottish Natural Heritage

1 Background

DNA technologies could allow major improvements in the breadth and efficiency of the monitoring carried out in terrestrial systems by environmental public bodies (Creer *et al*. 2016; Hänfling *et al*. 2017; Tang *et al.* 2018). This includes routine surveillance (e.g. pest/pathogen detection, monitoring protected species, *etc*.), environmental impact assessments prior to potential development, and filling evidence gaps to better understand the effectiveness of interventions. Exploration of DNA-based monitoring methods for operational use has generally involved relatively small-scale pilot studies, such as testing the potential to describe an assemblage in a specific habitat, or to detect an individual protected species (e.g. Tang *et al*. 2018). These projects are important for assessing feasibility and more generally for building end-user understanding and experience. To complement and help guide such projects, it is valuable to identify priority topics across organisations and applications. For example, if monitoring particular taxa is identified as a problem for several organisations, filling gaps in barcode libraries for these taxa might unlock many different applications. Similarly, projects that establish methods for particular sample collection techniques (e.g. soil cores, traps) could be employed across a range of monitoring needs. A better understanding of end-user priorities can also help researchers to ensure projects are as relevant as possible.

To identify key priorities across environmental public bodies, three stages are useful:

1) **Understanding the details of monitoring needs across organisations and taxa**. This should include information on the current approach, the required scale, drivers, constraints, and the need for improvement. Synthesising this information can then demonstrate where there are common needs and what any alternative/complementary method should provide.

2) **Assessing the feasibility and benefits of using DNA for the key monitoring priorities identified in (1).** In some cases, the potential benefits of using DNA will be greater than in others. For example, where the main constraint in monitoring is limited taxonomic expertise to identify large numbers of samples, DNA would likely represent an important option. Conversely, if collecting samples is a major constraint (e.g. due to access/safety) and the same samples still need collecting for DNA analysis, then the benefits of the technology would be more limited. There may also be some monitoring needs for which developing DNA-based methods is technologically more challenging than others.

3) **Identifying the barriers to using DNA for achieving the key monitoring priorities and agreeing activities and actions for resolving these.**

This project aims to complete (1), by collating and synthesising monitoring needs across environmental public bodies involved in terrestrial conservation, environmental management, and pest/pathogen surveillance. The intention is to include monitoring needs for individual species and for species assemblages. Related interests, in particular population genetics, are outside the scope of the project, but are noted on a more *ad hoc* basis. The project also addresses (2) and (3) to an extent, by considering the reasons for the constraints in current monitoring, although it is not intended to evaluate the technical problems and barriers associated with DNA-based methods. Similarly, the project is not intended to review or describe ongoing development and existing use of DNA-based methods (though such a collation would be valuable).

2 Methods

Information on terrestrial monitoring activities and needs was gathered from environmental public bodies. The project is funded by the Defra DNA Centre of Excellence (CoE) and so the main emphasis is on Defra group organisations, but additional input was obtained from devolved administrations (Table 1). A spreadsheet was initially circulated to all interested organisations to add monitoring priorities. To increase input, individual telephone interviews were also offered and arranged with representatives from several organisations. In some cases, this included several individuals from the same organisation, to ensure that as wide a range of taxa and applications were covered. Additional input on an earlier draft of this document was also provided by e-mail from several organisations.

Table 1: Organisations that provided responses on terrestrial monitoring needs and the general topic of interest. Note that the lack of a response on a particular topic does not reflect a lack of interest within an organisation. Additional organisations across the UK were also invited to provide input but a response was not received. In principle, 'Soils' overlaps with 'Invertebrates' and 'Fungi' but is included as a specific topic to reflect the division of interests and specialisms within organisations. 'Population genetics' is outside the scope of this project but was highlighted by several organisations and so is included for information.[1]Environment Agency interests are primarily in riparian areas, transient streams in relation to pollution. [2]Forest Research and Forestry England interests are specific to forests. [3]Input from Defra was provided in relation to plant health but not on biodiversity monitoring.

Theme	NE	EA[1]	FR[2]	FE[2]	Defra[3]	SNH	NRW
Fungi (conservation)	✓	✓	✓	✓		✓	✓
Invertebrates (conservation)	✓	✓	✓	✓		✓	✓
Soils	✓	✓	✓	✓		✓	
Mammals	✓		✓	✓		✓	
Reptiles	✓			✓		✓	
Bryophytes and lichens	✓			✓		✓	✓
Vascular plants	✓		✓	✓		✓	
Plant pests and pathogens	✓		✓	✓	✓		
General assessment of functions, effects of pressures and interventions *etc.*	✓	✓	✓	✓		✓	
Population genetics	✓		✓		✓	✓	

Priorities differed between themes and respondents and so interviews did not follow a set format. However, the general aim was to obtain information on current and required monitoring, surveys, and other evidence-gathering activities, and to examine the following for each monitoring application:

1. The main driver(s) for the monitoring activity.
2. The species and/or assemblages monitored.
3. The magnitude of current and required sampling (e.g. number of sites, number of samples per site).
4. The main constraints and problems in the current monitoring activity.
5. An overall assessment of the effectiveness of the current approach.

Although the main aim of the interviews was not to assess the feasibility of applying DNA in monitoring, several respondents were familiar with the methods. Brief views on possibilities and challenges were therefore also recorded, but more detailed assessment of technical problems and constraints is outside the scope of this project.

The Results section first gives a general overview of drivers, problems, and constraints in current monitoring, before examining these issues more specifically for each of the topics in

Table 1. The subsequent text is my own interpretation and does not necessarily represent the positions of the organisations interviewed.

3 Results

3.1 Monitoring Drivers

The driver for monitoring is important because it influences the level of confidence that is required for any alternative/complementary method. For example, high confidence is needed where monitoring has substantial direct economic or environmental consequences (e.g. detection of a quarantine pest or pathogen, Environmental Impact Assessment to inform if a development should go ahead). The main monitoring drivers discussed during interviews are described below.

3.1.1 Protected Site Condition Monitoring

Site Condition Monitoring involves surveying for individual species or assemblages (‘features’) that are designated on protected sites. Site Condition Monitoring is the driver for a large proportion of the biodiversity monitoring by conservation agencies (NE, SNH, NRW), and is required to varying degrees for different taxa. Data are used to assess the performance of protected sites and to evaluate and inform management. There is increasing recognition that new technologies should be more effectively incorporated into Site Condition Monitoring (JNCC 2019). However, although new methods may not need to replicate current approaches exactly, there must be evidence that results are robust and allow spatial and temporal comparisons (https://jncc.gov.uk/our-work/key-aspects-of-common-standards-monitoring-csm/). Some cross-validation is also usually valuable to understand any differences with previous methods.

3.1.2 Protected Site Notification

Protected site notification can involve establishing new sites or adding to the list of species or assemblages that an existing protected site is designated for. Site notification restricts the activities that can be carried out, whilst erroneous non-notification can affect the status of species and assemblages of conservation interest. As such, it is important that notification decisions are supported by strong evidence. New site notifications are probably most relevant for taxa that are currently under-represented in the protected site network. In addition to protected sites, Nature Recovery Networks are in the process of being developed (Crick *et al.* 2020). NRNs emphasise protecting and restoring wildlife across a network of connected sites. NRN details are being developed, meaning there is potential for new methods to be incorporated into both the initial baselining (e.g. site identification) and the subsequent monitoring.

3.1.3 Protected Species Monitoring

Article 17 of the EU Habitats Directive requires the status of all designated species to be reported on every six years. For the UK, 77 terrestrial taxa are listed, covering vertebrates, invertebrates, plants and lichens (https://jncc.gov.uk/our-work/article-17-habitats-directive-report-2019-species/). At UK level, reports on these taxa include information on range, population size and trends, habitat availability, and future prospects, which are combined into an assessment of conservation status. The approach to monitoring varies across species but needs to provide sufficient within-taxon consistency to allow changes over time to be reliably assessed.

3.1.4 Environmental Impact Assessment

Environmental Impact Assessments are required prior to development. The depth of these assessments varies by taxa, with some posing a greater challenge than others. The consequences of assessment are similar to site notification in terms of restricting/not restricting activities and the resulting economic or environmental implications, and so again it is important that decisions are supported by evidence that is robust to challenge.

3.1.5 Detection of Pests and Pathogens

Of the organisations interviewed, pest and pathogen detection were primarily discussed in relation to trees. However, wider consultation would likely highlight interests in other plant pests/pathogens, as well as in other systems (e.g. livestock). Detection can result in statutory action such as destruction or quarantine of material. As such, it is important to avoid false positives and to understand whether detections represent viable organisms of the target species. Equally, false negatives can allow establishment and spread, and so are potentially also costly.

Only a small number of responses highlighted monitoring for other invasive non-natives as a concern, although again this at least partly reflects the interests of the interviewees. The use of DNA for detecting terrestrial INNS could be explored further if there is demand

3.1.6 Evaluating Interventions

The resources invested in conservation interventions are sometimes considerably larger than investment to monitor the effectiveness of these interventions (e.g. Geijzendorffer *et al.* 2015), and there are a number of areas in which evaluation is important. For example, payments are currently made to farmers adopting measures that are intended to benefit biodiversity. There are ongoing programmes to assess the effectiveness of these agri-environment measures (e.g. Cole *et al.* 2019; Emmett *et al.* 2019), and in future monitoring could link payments to results (https://www.gov.uk/government/publications/results-based-agri-environment-payment-scheme-rbaps-pilot-study-in-england). Such evaluation needs to provide data that allow reliable assessment of trends over time and comparisons with areas that have not implemented particular interventions, and to expand the breadth of taxa and functions represented. Monitoring the effectiveness of other conservation interventions (e.g. habitat restoration, reforestation) also requires time series data, ideally with pre-intervention baselines. Importantly, whilst results need to be robust and reliable there is probably less of an established formal framework dictating what methods are acceptable for evaluating interventions than for e.g. monitoring protected species or site condition.

3.1.7 Indicators and Organisational Priorities

In addition to specific monitoring applications, each organisation has higher-level priorities. These tend to be general ambitions relating to e.g. natural capital, resilience, ecosystem functioning/health *etc*., and often require synthesising many lines of evidence. In several cases, indicators to assess progress towards these objectives are being developed/improved, for example:

- Defra 25 Year Environment Plan indicators (Defra 2019).
- Scotland Ecosystem Health indicators (https://www.environment.gov.scot/our-environment/state-of-the-environment/ecosystem-health-indicators/).
- State of Natural Resources Report (Natural Resources Wales 2016).
- Forest natural capital accounts (Forestry England 2019).

There may be opportunities for using novel and existing methods in combination to develop new indicators that improve the ability to track progress. New indicators would be less constrained by following pre-existing methods but do need to go through a rigorous quality-checking process and once established should provide results that are spatially and temporally comparable.

3.2 General Problems and Constraints in Current Monitoring

Resources sometimes constrain monitoring to such a degree that neither conventional nor DNA-based methods can be applied on a suitable scale. However, there are also many instances in which conventional monitoring is possible but limited by factors such as the time and costs involved, or by the availability of expertise. Understanding the extent to which these factors apply is important, because DNA-based methods are better placed to address some limitations than others.

3.2.1 Taxonomic Expertise/Difficulty in Identification

For several topics in Table 1, taxonomic expertise is expensive and/or in short supply, meaning monitoring is difficult, time consuming, or costly to implement at a sufficient scale. As such, for some taxa the volume of samples exceeds the availability of experts and/or funding. In these cases, DNA-based methods may be valuable because sample collection and DNA analysis generally does not require taxonomic expertise. There are also some taxa with ongoing ambiguity over identification (even amongst experts) which DNA can help to resolve. However, there are important caveats to greater use of DNA in overcoming this limitation in conventional monitoring:

- Sample collection sometimes requires understanding species ecology to inform the collection strategy (also see 3.2.3 below).
- Interpreting results from DNA analysis without the necessary ecological knowledge (e.g. which species are expected in the habitat) increases the risk that errors and anomalies go undetected.
- Over-reliance on DNA-based methods may erode taxonomic expertise and ecological knowledge and reduce the credibility of environmental public bodies when liaising with landowners.
- Taxonomic expertise is critical for developing DNA-based monitoring, e.g. through methods validation and contributing to barcode libraries.
- The supply of skills in DNA-based methods is also finite (particularly understanding of bioinformatics). As demand for this expertise grows, the availability of bioinformaticians could become a bottleneck in future unless adequate training is provided.

Greater use of DNA-based methods could therefore help address the identification problems in current monitoring (Deiner *et al.* 2017) but will also depend on retaining taxonomic expertise.

3.2.2 Obtaining Samples

The problem of sample collection is most pronounced in remote areas or where there is a safety risk. The ability of DNA-based methods to contribute in these cases depends on the sample collection approach. DNA surveys would primarily provide an advantage if it involves different field methods – e.g. requiring fewer site visits or sample points.

A related problem is that for some taxa particular life stages can be collected but not reliably identified without an additional step (e.g. to raise these individuals to adults). This step may introduce problems or extra costs, whereas DNA-based methods can identify any life stage.

Lastly, one key potential advantage of eDNA is the possibility of using the same sample for multiple purposes if all organisations are aware of the shared interest.

3.2.3 Finding Species

Where the monitoring target is a single species (e.g. rare or invasive species) determining true presences and absences can be challenging – particularly at low abundance. The usefulness of DNA in these circumstances varies on a case-by-case basis. DNA is less valuable for surveying species that require specialist knowledge to locate, and where it is destructive for rare species. Conversely, if non-destructive methods can be used for DNA analyses (e.g. collection of hair samples, droppings, water samples *etc.*), this may provide a more effective approach to surveying some taxa at low abundance.

3.3 Monitoring Needs for Specific Topics

Table 2 summarises priorities and key constraints across monitoring activities, and the subsequent sections provide more detail.

Table 2: Priorities and constraints in current terrestrial monitoring. Contents are my own interpretation of interviews and subsequent comments and so give an indication of key priorities but should not be treated as an exhaustive assessment of all monitoring needs across all organisations. Note that the potential limitations and constraints of DNA-based methods is not considered here. This table is also not intended to describe existing DNA work.

Topic	Key groups	End User Priorities	Need for improvement	Key constraints in current approach
Fungi (conservation). See 3.3.1	Assemblages. See Bosanquet *et al.* (2018) for species lists by habitat	Protected site designation	High	• Uses aboveground surveys. These depend on the presence of fruiting bodies, which only appear transiently and are spatially heterogeneous • Taxonomic revisions • Taxonomic expertise and difficulty in identification • Current method requires multiple site visits • Understanding of species ecology
		Protected site monitoring	Medium (but would increase with more site designations)	
		Evaluating interventions	Low-Medium	
	Mycorrhizae	Understanding forest and soil health, and air pollution	Medium	• Very little data being collected
	Single species. E.g. *Battarrea phalloides*, *Boletus reguis*; *Buglossoporus pulvinus*; *Hericium erinaceus*	Routine monitoring. Species detection	Low-Medium	• Variable, dependent on species
Invertebrates (conservation). See 3.3.2	All. Particularly Coleoptera, Diptera, and aculeate Hymenoptera. Also interest in including other taxa	Protected site monitoring	High	• Taxonomic expertise to identify large number of specimens • Interpretation if parts of an assemblage are increasing and parts are deteriorating • Site remoteness or safety considerations • Understanding of species ecology • Timing of trapping in relation to regional climate differences • Potential need to use multiple trap types
		Protected site designation	Low-Medium	
	All	Assessing effects of woodland management	Medium	As above
		Assessing effects of riparian restoration	Low-Medium	• N/A – valuable potential application

Topic	Key groups	End User Priorities	Need for improvement	Key constraints in current approach
Invertebrates (conservation) contd.	Pollinators	Understanding and mapping plant-pollinator interactions and link with pollination services	Low-Medium	• N/A – valuable potential application
		Evaluating agri-environment interventions	Medium	• Taxonomic expertise to identify large number of specimens
	Many individual species	Protected site monitoring	Low-Medium	• Variable, depending on species
Soils **See 3.3.3**	Assemblages (e.g. including springtails, mites, nematodes, earthworms, tardigrades *etc.*)	Assessing soil health Monitoring changes in soil biodiversity Evaluating agri-environment interventions Effects of woodland management	High	• Taxonomic expertise and understanding species ecology to design sampling and interpret results
Mammals **See 3.3.4**	Several semi-aquatic and terrestrial species of conservation importance (e.g. water vole, otter)	Protected site monitoring; Surveys for development	Medium	• Current method requires multiple site visits and at some sites can have safety risks
	American Mink	Monitoring to inform management	Medium	
	Hazel dormouse	Status of Article 17 species; Surveys for development	Medium	• Current method not very efficient
Reptiles **See 3.3.5**	Several individual species	Protected site monitoring; Status of Article 17 species; Surveys for development	Medium	• Current method requires multiple site visits and is expensive NB: Methods that use DNA as a complement to other approaches are being investigated
		Disease detection	Medium	• Not discussed extensively, but DNA likely to be part of solution
		Within-population genetic variation, understanding metapopulations and inbreeding	Medium	
Bryophytes and lichens. **See 3.3.6**	Individual species	Article 17 species; Protected site monitoring	Medium	• Differences in identification between different experts
Vascular Plants	Some individual species	Protected species monitoring	Low-Medium	• Accessibility of site • Difficulty in finding species

Topic	Key groups	End User Priorities	Need for improvement	Key constraints in current approach
Vascular plants (contd.). See 3.3.7	Flowering plants	Inform pollinator conservation	Medium	• Not stated
		Understand airborne pollen to inform public health	Medium	• Difficult to identify
Plant pests & pathogens. See 3.3.8	Several individual species (e.g. *Phytophthora*)	Reliable detection that avoids false positives and false negatives	Medium	• DNA already used for detection but may be possible to refine methods and/or develop more systematic surveys
	Vectors	Vector mapping to predict spread of pests and diseases	Medium	• General need for improvement and development, with DNA suggested as one way to achieve this
Biodiversity indicators and metrics. See 3.1.7	Any and all terrestrial taxa	Many, including national strategies (e.g. 25 Year Plan, Scottish Biodiversity Strategy), Natural Capital accounting, biodiversity status and trends for international reporting, agri-environment assessment, effects of pressures and interventions	Medium-High	• Monitoring costs • Sometimes ambiguity over monitoring requirements beyond general ambition • Potential need to combine with other techniques and knowledge to understand e.g. ecosystem functions, resilience *etc.*

3.3.1 Fungi (see Pests and Pathogens for fungal pathogens)

Interest in surveying fungi spans several organisations across a range of habitats, with relevant applications including protected site designation and monitoring, assessing the effectiveness of agri-enviroment interventions, and monitoring mycorrhizae to better understand forest health. This interest primarily relates to assemblage-level information.

Monitoring fungi using non-DNA methods has several important constraints, including **(i)** difficulty in identification, **(ii)** ongoing taxonomic revisions, and **(iii)** the requirement for multiple site visits that rely on detecting transient fruiting and only provide a partial picture of the belowground biota. The limited knowledge of the ecology and distribution of fungi also contributes to a circular situation in which relatively little is known and so little protection and monitoring takes place (e.g. only around 12 sites are designated for fungi in England and 8 in Scotland), meaning knowledge remains limited. This issue is addressed through the recently published SSSI guidelines for non-lichenised fungi (Bosanquet *et al.* 2018), which proposes lists of species of conservation interest for a range of habitats. In principle, this can be used for notifying fungal assemblages on protected sites and for subsequent monitoring, and Bosanquet *et al.* (2018) recommend future incorporation of DNA-based methods into SSSI designation and monitoring, noting several benefits – e.g. detecting cryptic species, confirming morphological identification, and providing information on species that rarely fruit. However, greater use of DNA will require establishing suitable baselines, because DNA-based methods using belowground sampling may give quite different communities from what is visible aboveground and positive detection does not necessarily indicate a viable organism is present. Suitable sampling design is also needed to account for spatial heterogeneity in fungal distribution belowground.

In addition to assemblage-level assessments, some individual fungi require monitoring, including those on Schedule 8 of the Wildlife & Countryside Act (e.g. *Battarrea phalloides*, *Boletus regius*, *Buglossoporus pulvinus* and *Hericium erinaceus*), and potentially others of conservation interest. The suitability of existing monitoring methods will differ for every species and is not discussed here, but the species of greatest interest are likely to be those that fruit sporadically – in such cases, DNA-based initial detection might be used to target confirmatory follow-up surveys.

Key Applications:

- Describing assemblages for protected site notification
- Describing assemblages for protected site monitoring
- Evaluating the effectiveness of agri-environment interventions.
- Describing mycorrhizal assemblages to assess forest health

3.3.2 Invertebrates (See also ‘Pest and Pathogens’ and ‘General Metrics’)

Assemblage-level invertebrate surveys are a priority for several applications and organisations. Protected site monitoring is particularly important, because over 1,000 sites are designated for invertebrate assemblages (approximately 800 in England, 200 in Scotland, and 85 in Wales) and require routine monitoring. In England, the Pantheon (https://www.brc.ac.uk/pantheon/) database defines 25 Specific Assemblage Types (SATs) associated with terrestrial, freshwater, and coastal habitats. Each SAT lists tens to hundreds of species across several taxa, and the presence or absence of these species is used to help understand site quality.

In practice, a smaller number of key groups identified in Pantheon are typically targeted by monitoring, rather than the entire assemblage, and Table 3 shows the highlighted taxa for SATs that have been notified on >50 sites in England. This emphasises monitoring

Coleoptera, Diptera, and Hymenoptera across a range of habitats. Collectively these groups are also specifically mentioned as features on around 30 high priority sites in Scotland and represented in general invertebrate assemblages that are notified features in many more. However, the emphasis on these Orders partly reflects a lack of knowledge of other groups rather than a lack of importance, and there is interest in a more taxonomically representative bulk-sampled community if this can be generated and the conservation relevance established. The range of sampling methods in Table 3 is also notable because some methods may translate more easily to DNA-based monitoring than others (e.g. depending on the numbers of specimens collected and specimen condition).

Table 3: Priority assemblages, key taxa, and number of sites for assemblages that have been notified on >50 protected sites in England. The table gives a general indication of the scale of need rather than precise information. Note that monitoring effort is also shaped by which sites are likely to change most rapidly. * Scrub heath and moorland is included because the actual number of sites with this assemblage isn't precisely established but is thought to be substantially more than shown.

Assemblage	Key taxa	Sites	Main survey method
Heartwood decay	Coleoptera, Diptera	59	Beating and targeted search. Sweep netting if considering Diptera
Fungal fruiting body	Coleoptera	59	Beating and targeted search. Sweep netting if considering Diptera
Bark and sapwood decay	Coleoptera, Diptera	60	Tubing/pooting, beating, targeted search. Vane traps as alternative
Rich flower resource	Hymenoptera	63	Spot sweeping
Open water on disturbed sediments	Aquatic fauna	66	Pond netting
Arboreal canopy	Not clear from Pantheon. Araneae, Coleoptera, Diptera, Hemiptera, Hymenoptera, Lepidoptera?	70	Not clear from Pantheon – fogging?
Open short sward	Coleoptera; Diptera; Heteroptera; Hymenoptera; Lepidoptera; Orthoptera	104	Ground searching, spot-sweeping, sweeping. Pitfall traps as an alternative
Bare sand and chalk	Coleoptera; Diptera; Heteroptera; Hymenoptera; Lepidoptera	128	Ground searching, spot-sweeping. Suction sampling as an alternative
Scrub heath and moorland*	Araneae; Coleoptera; Diptera; Hymenoptera	26	Spot-sweeping, ground-searching sweep-netting. Pitfall traps and suction sampling as alternatives

Other important invertebrate monitoring priorities include new site designations (particularly in under-represented areas such as uplands), evaluating agri-environment interventions, assessing the effects of woodland management, evaluating riparian restoration, and monitoring transient waterbodies. The proposed Nature Recovery Networks (NRNs) will also require invertebrate surveys and monitoring, and there may be potential to incorporate new methods into both the initial baselining (e.g. site identification) and subsequent monitoring. Interest in assemblage-level invertebrate monitoring therefore spans a range of applications and organisations.

Resources are an important general constraint in invertebrate monitoring and affect some organisations and applications particularly severely. For organisations able to carry out some level of invertebrate monitoring, the availability of contractor expertise was frequently highlighted as a major problem and bottleneck. Several other limitations in current monitoring were also noted – DNA-based methods will be better suited to resolving some of these limitations than others:

- Weather dependency of surveys (especially in more northerly and higher elevation areas).
- Risks of false negatives if sampling cannot be carried out effectively enough in the time available.
- Record verification if no specimen has been collected.
- Interpreting cases where some components of an assemblage are increasing but others are deteriorating.
- Site access due to remoteness or safety considerations.
- Lack of ecological knowledge of some invertebrate taxa.

DNA-based methods are probably most valuable for describing invertebrate assemblages where large numbers of specimens are collected and/or identification is challenging. This could apply to routine monitoring and to assessing the effects of interventions. End user requirements are more flexible for the latter, but routine monitoring can still incorporate new methods provided these are calibrated to existing data and regulations. Even where gaps in barcode libraries mean that DNA can only identify organisms to the level of Operational Taxonomic Units, consistent OTUs may still be valuable for sampling currently unrepresented biodiversity across different habitats.

Monitoring is also important for many individual species (e.g. in Wales there are 165 invertebrate species representing 398 features on 160 SSSIs). The effectiveness of this monitoring differs on a case-by-case basis and is not discussed further here but could be considered in a more detailed follow-up.

Key Applications:
- Site Condition Monitoring (particularly for Coleoptera, Diptera and Hymenoptera?).
- Evaluation of agri-environment interventions and other management/restoration.
- Potential use in future Nature Recovery Network monitoring.

3.3.3 Soils (also see fungi)

There is no formal soil biodiversity monitoring requirement, and most soil fauna is very poorly understood both taxonomically and ecologically. This limited understanding contributes to a lack of protection, which in turn means there are few resources to improve understanding and so monitoring remains limited. Lack of soil monitoring is a critical gap for two reasons. Firstly, soil biota influence many key ecosystem processes such as nutrient cycling and soil aeration (Orgiazzi *et al.* 2016). Secondly, given that most well-studied aboveground taxa underwent major declines during the 20th Century, soil biodiversity is also likely to have been severely affected. However, the lack of monitoring means that biodiversity loss belowground has been hidden and largely unquantified, with little remedial interventions.

Improved information on soil health, functioning, and biodiversity is increasingly being prioritised as part of agri-environment evaluation and monitoring as well as for other assessments of the effect of land management, pollution, *etc*. Ongoing research to develop national-level soil monitoring (e.g.http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=2&ProjectID=20276) and to inform agricultural interventions (https://gtr.ukri.org/projects?ref=102272) is helping to address this. Soil monitoring is less constrained than some other activities by the need to follow previous approaches, although consistency with related soil surveys such as Glastir agri-environment monitoring (Emmett *et al.* 2017), and the Europe-wide Land Use and Coverage Area Frame Survey (LUCAS; Orgiazzi *et al.* 2018) could increase the value.

Although DNA will almost certainly be important in soil biodiversity monitoring (Arribas *et al.* 2016), demonstrating comparability of outputs with conventional identification remains important for routine operational use. If part of large-scale long-term monitoring then spatial and temporal consistency is needed, whilst a higher level of robustness might be required if linking soil data to agri-environment payments.

A further area of interest for soils is identifying antimicrobial resistance. This probably requires more specific exploration than can be given here, in order to link with ongoing work by other CoE organisations (e.g. APHA).

Key Priorities:

- Development of soil monitoring methods that describe soil community biodiversity and soil health and can show how systems are responding to changes (note that it may be possible to adapt existing methods from other soil surveys).
- Additional DNA-specific priorities highlighted in responses:
    - Understanding the extent to which different DNA-based soil monitoring methods produce different results and why.
    - Broad-scale comparison between conventional identification and metabarcoding across habitats and over time.
    - National scale testing of primers and amplification.
- Detecting antimicrobial resistance.

3.3.4 Mammals

Mammal monitoring has tended to be for individual species rather than assemblages (although camera trapping provides opportunities to survey for several species simultaneously) and covers a range of purposes:

i. Assessing the status of individual species.
ii. Surveys prior to development.
iii. Detecting invasive mammals to inform management.
iv. Disease monitoring.
v. Understanding diet.

For i-iii, the current (non-DNA) approach is adequate in several species, but there are instances where improvements would be valuable. Firstly, many mammals require multiple site visits to confirm presence/absence – this applies to conservation assessments, surveys prior to development, and surveys for invasive species such as mink (particularly where these are the focus of control programmes or are potentially colonising new areas). Surveys can be labour intensive and pose safety risks at certain sites, and also apply across a relatively large number of locations because of the distribution, home range, and dispersal capabilities of target species. DNA-based methods could therefore be valuable, particularly if these simultaneously detect multiple semi-aquatic and terrestrial species (Sales *et al.* 2020). Secondly, hazel dormouse monitoring was specifically highlighted as a difficulty. Hazel dormouse is a protected species that is widely distributed but at low densities. Although citizen science monitoring through the National Dormouse Monitoring Programme provides a good overall picture, other surveys (e.g. for development) are expensive and not necessarily effective, so more efficient methods would be valuable. Lastly, disease monitoring and understanding diet and predator-prey interactions in mammals was discussed more briefly but is also potentially of interest.

Some responses noted obstacles to using DNA for some applications. For example, where sites are in more remote areas, the survey costs may be high irrespective of the method and it has been challenging to find the right systems to test the effectiveness of eDNA for early detection of mink and to confirm eradication. In practice therefore, eDNA and other methods such as camera trapping are likely to be complementary.

Key Priorities:
- Semi-aquatic mammal monitoring.
- Hazel dormouse monitoring.
- Detecting diseases in mammals.
- Dietary analyses.

3.3.5 Reptiles and Amphibians[3]

The main focus of current monitoring is for individual protected species. The approaches used require multiple visits (sometimes at night) and are expensive and could be more effective. Alternative methods are already being explored, particularly involving working with NGOs (Amphibian & Reptile Conservation Trust, Froglife) to develop citizen science monitoring. This would likely use multiple methods, with DNA potentially contributing (e.g. grass snake detection using eDNA in water to assess status in specific low-density areas). DNA is also potentially valuable for other conservation applications, specifically, testing for chytrid fungus and other diseases and examining inbreeding levels and metapopulation structure.

Key Priorities:
- Detecting individual protected species.
- Disease detection and understanding population structure.

3.3.6 Bryophytes and Lichens

A relatively large number of protected sites are designated for bryophytes and/or lichens (e.g. in Scotland around 70 in each case). There is also a general need to assess the effectiveness of restoration and management (e.g. in bogs). Current methods gather some useful information but are imperfect. For lichens, detecting gradual change is difficult, and even expert surveyors can disagree on identification. In bryophytes, aboveground conservation assessments sometimes only provide a partial picture because these do not include belowground diaspores (Callaghan *et al.* 2020). More consistent identification and more comprehensive monitoring are therefore the main areas of interest. Despite the limitations of current methods however, the feasibility and usefulness of DNA may also be constrained e.g. because expertise is still needed to find the target organism(s), and because destructive sampling that damages populations may sometimes be needed. In addition, Callaghan *et al.* (2020) suggest that incomplete barcode libraries pose a substantial problem if species names are needed.

A more speculative potential opportunity for monitoring involves air sampling to detect spores. This might provide valuable information across a site, although substantial validation and ground-truthing is needed to understand the area that is being sampled, the effects of weather conditions, spore viability, *etc*., as well as how this relates to data collected through conventional means.

[3] Use of DNA in monitoring amphibians is already comparatively well-developed, so this was not considered extensively.

Alongside assemblage-level monitoring, some individual species are of interest, e.g. *Petalophyllum ralfsii*; *Buxbaumia viridis*; *Hamatocaulis vernicosus*. In Scotland, *B. viridis* can have an economic impact because as a protected species it can restrict timber harvesting if present, whilst more rapid surveys for *H. vernicosus* would be helpful.

Key Priorities:

- More consistent lichen identification to reduce variability between surveyors.
- Some interest in surveying for particular species and in diaspore banks in soils.
- Possible use of air sampling as an alternative method.

3.3.7 Vascular Plants

Routine vascular plant monitoring by conservation agencies tends to be relatively effective and introducing DNA-based methods was not viewed as urgent in relation to protected species and sites. However, pollen identification may attract greater interest. This can help understand flower visitation and inform pollinator conservation. Improved pollen identification surveillance could also contribute towards pollen forecasting to guide public health measures (Brennan *et al.* 2019).

Where there is a need for vascular plant monitoring from conservation agencies, this tends to be for detecting particular species rather than whole communities, and so requires some level of expertise. Key potential interests are individual species that are difficult to consistently detect (e.g. ghost orchid) and plants in dangerous or remote locations. In the former case, alternative methods may be beneficial provided they are not destructive and reflect the presence of a viable organism. However, DNA would probably not provide major benefits where site accessibility is the main problem (although alternative methods might allow samples to be taken by non-specialists – again, providing this is not destructive).

Key Priorities:

- Pollen identification (e.g. to inform pollinator conservation, public health).
- Improving monitoring on remote or difficult sites.
- Methods for detecting a small number of individual priority species.
- Potential re-use of samples collected for other purposes (e.g. testing eDNA from waterbodies for slender naiad).

3.3.8 Plant Pest and Pathogen Monitoring

DNA is already an important tool for detecting and monitoring several plant pests and pathogens. However, there are shared concerns and interests with other topics (e.g. the availability of invertebrate taxonomic expertise). There are also several areas of interest for improvement. For some species, these are technical, such as developing and refining protocols. But in many others the focus is more on improving the operational process and the evidence. For example, there is interest in developing more systematic sampling for *Phytophthora* and integrating this with alert systems. Similarly, there are several outstanding questions that require molecular techniques and that will help to better manage pest and disease outbreaks, including:

- Understanding the origins and genetics of pests and pathogens (e.g. *Cryphonectria parasitica*, pine tree lappet moth, pinewood nematode).
- Identifying potential biological control agents (e.g. parasitoids).
- Contributing to UK-wide vector mapping to better predict potential pest/pathogen spread.

Lastly, there is also interest in the use of broader population genetics techniques within plant health (potentially e.g. understanding adaptive capacity, presence of disease-resistant individuals, *etc.*). Whilst this is outside the current core scope of the Centre of Excellence, further consideration may be valuable.

Because of the comparatively advanced status of DNA in monitoring pests and pathogens, the area was not investigated as extensively as other topics. However, more specific exploration of potential cross-organisation priorities could be carried out subject to interest.

4 Synthesis and Recommendations

The responses illustrate the breadth of terrestrial monitoring but also show many interests and applications that are shared across organisations. Topics of particular importance for improved methods include:

1) **Assemblage-level invertebrate monitoring methods**
   This is a priority for conservation agencies, and for the Environment Agency (particularly in riparian strips), and Forest Research and Forestry England (in woodlands). There may also be some overlap with monitoring for invertebrate pests (Defra Plant Health, Forest Research). Although specific needs differ, the general approach is similar across habitats and organisations: various sampling methods that then require physical specimens to be identified. Large numbers of specimens are often collected and taxonomic expertise is often a constraint. DNA methods that reliably characterise the invertebrate assemblage and are comparable with previous data would therefore represent an important benefit for multiple organisations and applications. These could be explored based on - e.g. collection method, taxon, or habitat. Cross-organisation discussion could help to focus this exploration on common priorities (e.g. by agreement on which taxa are most useful to fill barcode gaps for).

2) **Assemblage-level fungi monitoring**
   This is an important gap in current monitoring for conservation agencies and is relevant for organisations interested in more general biodiversity metrics (e.g. Forestry England). The gap reflects the limitations of aboveground survey methods and the problems posed by taxonomic revisions. DNA-based methods could help circumvent these issues. Improved methods would not necessarily represent a cost-saving but are needed to better understand and conserve this important, poorly characterised component of UK biodiversity. However, some potential issues with DNA-based methods in fungal monitoring have already been identified (e.g. spatial sampling structure, establishing reference states. These issues could be priorities to address collaboratively).

3) **Soil biodiversity**
   There is very little soil biodiversity monitoring, but this is a need highlighted across several organisations and encompassing national monitoring and assessing interventions (e.g. woodland management, evaluating agri-environment monitoring). The requirements are less clear than for some other applications with defined species lists but given the taxonomic constraints in relation to soil biodiversity, DNA is likely to be important in both basic understanding and in routine monitoring. Any developmental activities in this area should link with related soil assessments (e.g. potential national soil monitoring programme, LUCAS soil biodiversity surveys).

4) Semi-aquatic and terrestrial mammals
This was not discussed as extensively as some other priorities but was highlighted by several organisations. If DNA-based methods can provide reliable assemblage-level data that reduce the need for multiple species-specific surveys, this could have cost/efficiency savings. There is particular interest in the potential for eDNA from waterbodies to provide more comprehensive information (e.g. through detection of semi-aquatic and terrestrial mammals, use of citizen science *etc.*).

5) Interventions, pressures, natural capital assessments
In addition to taxon-specific priorities, many organisations require more general information on how biodiversity varies spatially and temporally (e.g. to assess the effects of pressure and interventions, monitoring natural capital, provide comprehensive information on biodiversity status and trends, *etc.*).

4.1 Proposed Next Steps

For topics 1-4, proposed next steps are:

1) Short follow-up discussion amongst end-users with an interest in the particular topic (e.g. terrestrial invertebrate assemblages, fungal assemblages *etc.*) to gauge demand for subsequent collaboration.

2) Subject to interest, workshops or telecons bringing together end-users and academics to identify the barriers to operational use of DNA for each topic and propose specific activities. If there is a consensus list of activities, these would potentially **(i)** provide a stronger case for funding, **(ii)** allow more strategic investment in key projects, **(iii)** identify areas for cross-organisation collaboration, and **(iv)** help researchers and end-users to co-develop relevant projects (e.g. studentships). Workshops could take place under the auspices of the UK DNA Working Group.

To a degree these steps also contribute to topic (5). However, any more specific work on this topic would be most useful in combination with the related DNA Centre of Excellence project on developing new metrics

4.2 Other Areas of Interest

The above prioritisation omits several other areas of interest. Further consideration of demand is needed, but a brief discussion is given below:

- Dormouse monitoring and mammal diseases
  Could be incorporated within discussions relating to mammals

- Reptile monitoring (including diseases and metapopulation structure)
  Comparatively advanced and has already received funding from other sources so may not need specific input? However, liaison could help to identify potential future projects and provide learning opportunities

- Plant pests and diseases
  Comparatively advanced and no indication that support in prioritisation is needed? However, support for activities would help improve evidence base (e.g. vector mapping, Phytophthora alerts). Also, potential opportunities for collaboration in sample collection and to use experience in methods development and validation

- Bryophytes and lichens
  Potential need for development of some DNA-based monitoring methods. Could be considered further if there is interest across several organisations, e.g. in relation to air sampling methods.

- Vascular plants
  Potential for high interest in pollen monitoring. Possible DNA applications for aquatic plants (including opportunities for re-using samples collected for other purposes). Could be considered further if there is interest across several organisations (e.g. in relation to pollen)

- Other single species monitoring
  There are a relatively large number of individual species for which some degree of monitoring is required. It is not possible to discuss each of these individual cases. However, some species are in taxa that are addressed by the priorities in 1-4. For other species, more focused consideration of the feasibility of DNA-based methods would probably be most efficiently done on a case by case basis according to demand.

5 Conclusions

There are several substantial areas in which current terrestrial monitoring could be improved, and a number of similar priorities across organisations. Superficially, many of these needs are amenable to DNA-based methods. However, as with the incorporation of any novel method into operational monitoring, validation work is required – e.g. cross-comparison with data from conventional surveys to ensure comparability, agreeing protocols, adoption into regulatory framework, *etc*. The incompleteness of reference libraries will also pose a problem for some applications.

The information presented here could help to inform where to target research and development (e.g. methods development, prioritisation of barcode library gap-filling being planned as part of a related CoE project; Price *et al.* 2020). Important next steps could involve follow-up discussions and potential workshops on priority topics involving end-users and academics. Topic areas could also help researchers and end-users identify and co-develop relevant projects (e.g. as part of studentships).

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