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Provision of statistical advice to the Marine Protected Sites monitoring project

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Summary

Species diversity per biotope was investigated for two case studies. Species accumulation curves were constructed to estimate the number of stations required to achieve a given coverage of the total number of species present.

Case study 1: Rock Unique[1]

These data are based on sediment samples from 46 stations, covering four sediment types, namely coarse sediment (12 stations), mixed sediment (21 stations), mud (2 stations) and sand (11 stations). A total of 413 entries (rows in species x station matrix) were obtained, 338 of which were recorded as counts with the remainder recorded as presence or absence. In addition, 306 entries were also recorded as biomass. Two-thirds of these entries were identified to the species level, and this went up to 82% identified to at least the genus level and 93% identified to at least the family level. These data were analysed as:

- incidence data (this includes abundance data, with counts converted to presence or absence);
- abundance data; and
- biomass data

The incidence and abundance data analyses indicate that the observed number of species per sediment is approximately 57 to 77% of the estimated total number of species per sediment.

In order to sample 80% of the estimated total number of species per sediment type, based on incidence data, 22 and 29 stations would be required for the coarse sediment and sand, respectively, whereas for the mixed sediment 37 stations would be needed. Analysis of the abundance data yields similar results, with 18 and 22 stations for the coarse sediment and sand, respectively, and with 41 stations for the mixed sediment.

Various measures of diversity were investigated. Species richness looks at the total number of species, whereas Shannon index and Simpson's index take account of the total number of species as well as how evenly the counts are distributed among species. To account for the complexity of the phylogenetic tree, phylogenetic diversity and taxonomic diversity were also investigated. Phylogenetic diversity (PD) is given as the total number of branches in the tree, whereas taxonomic diversity looks at either the sum of the path lengths connecting pairs of species (TDsum) or at the average path length connecting pairs of species (TDavg). Species richness and PD are based on incidence data, whereas Shannon and Simpson are based on abundance data. Taxonomic diversity can be based on either incidences or abundances. Despite these differences in interpretation, the diversity patterns observed were similar, i.e. the methods agreed on which stations were regarded as most diverse. Furthermore, phylogenetic diversity and taxonomic diversity (TDsum) showed a strong association with the total number of species observed, and diversity patterns were similar irrespective of whether diversity was calculated based on incidence or abundance data.

Ordination techniques showed similar patterns for incidence and abundance data. Patterns were also similar irrespective of whether data were identified to the species level, genus level or family level (despite the genus and family levels containing more data). The stations tended to cluster according to sediment type, suggesting that sediment type has a large

[1] Rock Unique was designated as an MCZ in December 2013. It's name has been changed to North East of Farnes Deep

effect on community structure. Stations that share the same sediment and are in close proximity geographically also tended to cluster in the ordination plots.

[2]Case study 2: Solan Bank

These data consist of stills obtained from 23 stations, covering eight biotopes. All data were recorded as incidence data. Of the total number of entries, 77% was identified to the species level, 84% was identified to at least the genus level and 91% was identified to at least the family level. Three of the eight biotopes contained one station each and for these biotopes it was not possible to estimate the total species richness. For the remaining five biotopes, the observed number of species was approximately 67 to 88% of the estimated total number of species per biotope.

In order to sample 80% of the total number of species, it is estimated that five to nine video stations per biotope are needed, assuming that 2-4 stills per station are obtained. The exception was the offshore coarse sediment habitat (SS.SCS.OCS) which would require 21 stations to sample 80% of the total number of species. For the biotopes that were observed at one station only it was not possible to perform these calculations.

Phylogenetic diversity was calculated for each biotope and also for each station and each still. The diversity per still covered, on average, two-thirds of the diversity observed per station. The diversity per station covered about half the diversity of the corresponding biotope for the two CR.MCR biotopes. For the offshore coarse sediment habitat (SS.SCS.OCS), the diversity per station was approximately 25% of the biotope diversity.

Ordination techniques were used to graphically display similarities between stations or stills. In either case, the samples tended to cluster according to assigned biotope. These patterns were similar for data identified to the species level, genus level and family level (despite the genus and family levels containing more data). For stations covering more than one biotope, the species composition tended to be different among these biotopes, despite the stills being obtained in close geographical proximity. Ordinations based on the stills data tend to show separation of stills according to biotope. Furthermore, stills obtained from the same station and the same biotope may have quite different species composition, indicative of a single still not covering the full species diversity of that station.

Are biotopes (Solan Bank) less diverse than sediment types (Rock Unique)?

For those biotopes and sediments that had 10 or more stations each (namely Solan Bank biotopes CR.MCR.EcCr.CarSp.Bri, CR.MCR.EcCr.CarSp.PenPcom and SS.SCS.OCS, and Rock Unique sand, coarse and mixed sediments) we find that the number of observed species per Solan Bank biotope ranged from 53 to 70, whereas for the Rock Unique sediments it ranged from 152 to 209. This is probably a reflection of sediments being composed of more than one biotope and hence increasing species diversity, although the SS.SCS.OCS habitat at Solan Bank was also likely to be composed of more than one biotope. The average number of species per station, however, was similar for the Solan Bank biotopes (12 to 41) and Rock Unique sediments (21-37).

Association between diversity and sediment type

Relating the ordination plots to sediment composition showed an association with % bedrock obtained through visual observations for the Solan Bank data. For the Rock Unique data, an association with the % sand and gravel obtained through particle size analysis was observed. In addition to relating ordinations to sediment composition, we also looked at correlations between diversity indices and sediment composition. For the Solan Bank data this showed a positive association between diversity and % bedrock, boulders, and cobbles, and the negative association with %sand. For the Rock Unique data and positive association was seen between diversity and % gravel, and a negative association with %sand. Although

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these associations were statistically significant, the strength of these relationships was relatively weak with no more than 10 to 25% of the variation in diversity explained by the sediment characteristics.

Sampling schemes

Sampling schemes can generally be divided into random sampling, systematic (grid) sampling, and stratified sampling. When the habitat (i.e. sediment or biotope) distribution among the site is known, stratified sampling is preferred. This way each habitat will be targeted specifically at this will ensure that sufficient samples per habitat are obtained.

Contents

1 Introduction

1.1 Background

The Joint Nature Conservation Committee (JNCC) is working to develop monitoring plans for its offshore Marine Protected Areas (MPAs). These MPAs are characterised by features such as the type of habitat (e.g. circalittoral rock, infralittoral rock, sand, mixed sediment, coarse sediment). Future monitoring surveys will aim to address whether the designated features within an MPA are in favourable condition. For this contract, data was provided from previous surveys whose purpose was to delineate the presence and extent of the features proposed for designation. Within the survey area, each habitat type has one or more sampling stations, from which data have been collected. Two case studies were chosen to examine as part of this contract: Rock Unique[3] proposed Marine Conservation Zone (MCZ), which is being proposed for its broadscale sedimentary habitats, and Solan Bank candidate Special Area of Conservation (SAC), which is being designated for its rocky reef features. Different sampling techniques were used in each site; a Hamon grab was used to collect infaunal samples at Rock Unique while a drop camera was used to collect images of epifauna at Solan Bank cSAC. Data for both sites were made available to the Contractor for data exploration.

1.2 Aims and Objectives

Based on data from Rock Unique and Solan Bank, the main aims are to

- identify trends in species diversity; and
- explore options for sampling strategies for baseline monitoring surveys.

The main questions to be addressed in the proposed work are:

A. Explore the suitability of various metrics that summarise species abundance and diversity;
B. Investigate whether the existing data show any spatial variation in species abundance and diversity;
C. Investigate whether the existing data show any trends or patterns; and
D. Assess whether the existing data contain sufficient information to estimate appropriate sample sizes for a monitoring baseline survey.

Questions B and C will be addressed for each case study and each phylogenetic hierarchical level (species, genus, family etc) separately, using exploratory data analysis approaches.

Question D will be addressed for each case study for each habitat type.

Question A is of a more general nature and is not restricted to case study or habitat type.

[3] Rock Unique was designated as an MCZ in December 2013. It's name has been changed to North East of Farnes Deep

2 Methodology

Based on phylogenetic classifications supplied by JNCC, the following hierarchical levels were distinguished: kingdom, phylum, class, order, family, genus, and species. Not all organisms were identified to the species level and therefore some of the results will be presented for data specified to species level, or data specified to at least genus level (this includes data specified to species level), or data specified to at least the family level (this includes data specified to genus level or species level) etc.

Where organisms were identified as 'juvenile' or 'female', this information was ignored and these entries were combined with other data from the same species.

For organisms belonging to the gastropoda or polychaeta class, the hierarchical level did not always include details on 'Order'. To be able to include these organisms in phylogenetic diversity calculations an artificial order was created where it was assumed that the order was the same as the family.

2.1 Metrics that summarise species abundance and diversity

Species diversity reflects the effective number of species found in a community. One of the simplest measures is species richness, which is the total number of species observed or the estimated total number of species. The latter attempts to account for the number of unobserved species. Species evenness refers to how evenly the numbers of individuals are spread over species. Diversity indices try to combine evenness and richness into one index. Such indices may be based on just the numbers of individuals spread across a number of species (e.g. Shannon, Simpson), or may take into account the complexity of the phylogenetic tree (phylogenetic diversity, taxonomic diversity).

2.1.1 Species richness

Estimation of total number of species

The total number of species observed in our sample depends on sampling effort, as smaller samples tend to contain fewer species. Methods exist that allow us to estimate the (unobserved) total species richness based on the observed number of species, plus an additional quantity. This additional quantity is an estimate for the number of unobserved species, and is based on the number of rare species observed in our data. One of the most widely used estimators is the Chao index (Chao, 1987).

For abundance data, it is defined as

$$S_p = S_0 + 0.5\, a_1 \frac{a_1 - 1}{(a_2 + 1)}$$

where S_p is the estimated total number of species, S_0 is the observed number of species, a_1 is the number of species in our data for which we have a count of 1, and a_2 is the number of species in our data with a count of 2. The species richness S_p can be calculated for each station separately or for the data from all stations combined, in which case it will give the species richness for the entire site.

For incidence data it cannot be calculated for each station separately but it can still be obtained for the site as a whole, as follows:

$$S_p = S_0 + 0.5 \frac{a_1^2}{a_2}$$

where a_1 is the number of species in our data that are observed at 1 station only, and a_2 is the number of species in our data observed at 2 stations.

Species accumulation curves and number of stations needed to achieve a given species coverage

Although the above methods provide us with an estimate of the total number of species present at a site, they do not provide any information as to how many stations we would need to observe at least 80% (say) of the total number of species. This is where species accumulation curves come into play.

Species accumulation curves (also called rarefaction curves) plot the number of species observed against sampling effort, which in our case is the number of stations sampled. Based on the data, the average number of species observed for a given number of stations is calculated (e.g. if we have 46 stations in total, say, then the average number of species observed if we would have had only 10 stations is obtained from looking at all possible combinations of 10 stations and the associated numbers of species observed for each of these combinations). Graphs can be constructed showing how the observed numbers of species increase with the number of stations (see for example Figure 1 in Section 3.2). Extrapolating these curves will show us how many stations will be required to cover at least 80% (say) of the total number of species. In the current report we will use a Michaelis-Menten formulation, which corresponds to rapid increase in species observed when we add a station to a small number of stations, whereas the increase in the number of species observed will be much less when we add an extra station to a large number of stations. The formulation is

$$y = Y^* \frac{S}{K + S}$$

where *y* is the average number of species observed when we have S stations, and *Y** is the estimated total number of species at the site. *K* is a parameter reflecting the number of stations needed to sample, on average, 50% of the total number of species. If we define *c* to be the percentage of species included in our sample, it then follows from rearranging the above that the corresponding number of stations needed to achieve this coverage is given as

$$S = \frac{K\, c}{100 - c}$$

It is important to keep in mind that this calculation tends to be driven by extrapolation beyond the observed number of stations, and as such the estimated number of stations required to achieve a notional 80% coverage, should be seen as indicative only.

2.1.2 Diversity indices: Shannon and Simpson

Diversity indices attempt to summarise how evenly individuals are distributed over species; the more even this distribution, the higher the diversity. The two most commonly used indices are Simpson's index and Shannon's index, both of which are based on p_i, which is defined as the proportion of the total number of individuals belonging to species *i*.

Simpson's index

Let $D = \sum p_i^2$, then Simpson's index is calculated as 1-*D* (Simpson, 1949). It is also known as the Gini-Simpson index. Frequently used is the inverse-Simpson index, which is 1/*D*, i.e.

$$\text{inverse-Simpson} = 1/\sum p_i^2$$

For one species only, the inverse-Simpson index takes on the value 1, whereas with increasing numbers of species and individuals which are evenly distributed over the species the inverse-Simpson index will approach infinity. In the current report we will use the inverse-Simpson index (but for convenience, and according to common usage, we will refer to this as Simpson's index).

Shannon index (also known as Shannon Wiener, Shannon Weaver, Shannon entropy)

Shannon's H (Shannon, 1948) is defined as

$$H = -\sum p_i \, LN(p_i).$$

It is a measure of uncertainty or unpredictability, i.e. if we take a new individual from our population (without knowing what it is), how predictable is it in terms of which species it will belong to? The larger this unpredictability, the larger the Shannon index. If we know we only have species A, then for a new individual of unknown species we are certain it has to belong to species A; there is no unpredictability and therefore Shannon's index = 0. As with the inverse Simpson index, when we have an increasing number of species and individuals are evenly distributed over species, the Shannon index will tend to infinity.

2.1.3 Example of Simpson and Shannon indices

Table 1 below gives examples of Shannon's and inverse Simpson indices, where it is assumed that we have 100 individuals belonging to up to 5 species.

Table 1. Examples of Shannon and Simpson's indices for 100 individuals belonging to up to five species (denoted as species A, B, C, D, and E).

# Species	Scenario	Species					Diversity Index	
		A	B	C	D	E	Shannon	Simpson
1 species	1	0	0	0	0	100	0.00	1.00
2 species	2	0	0	0	1	99	0.06	1.02
	3	0	0	0	5	95	0.20	1.10
	4	0	0	0	10	90	0.33	1.22
	5	0	0	0	20	80	0.50	1.47
	6	0	0	0	30	70	0.61	1.72
	7	0	0	0	50	50	0.69	2.00
3 species	8	0	0	1	9	90	0.36	1.22
	9	0	0	5	5	90	0.39	1.23
	10	0	0	1	19	80	0.54	1.48
	11	0	0	10	10	80	0.64	1.52
	12	0	0	1	1	8	0.64	1.52
	13	0	0	10	50	40	0.94	2.38
	14	0	0	33	33	34	1.10	3.00
4 species	15	0	1	1	1	97	0.17	1.06
	16	0	10	10	10	70	0.94	1.92
	17	0	5	5	45	45	1.02	2.44
	18	0	10	10	40	40	1.19	2.94
	19	0	1	33	33	33	1.14	3.06
	20	0	10	30	30	30	1.31	3.57
	21	0	25	25	25	25	1.39	4.00
5 species	22	1	1	1	1	96	0.22	1.08
	23	2	2	2	2	92	0.39	1.18
	24	5	5	5	5	80	0.78	1.54
	25	10	10	10	10	60	1.23	2.50
	26	5	5	30	30	30	1.38	3.64
	27	1	24	25	25	25	1.43	4.08
	28	20	20	20	20	20	1.61	5.00

The indices are lowest when we have only one species, and highest when we have 20 individuals per species. Generally, the indices increase when the distribution of individuals over species becomes more even, and when we observe more species. Furthermore, as these indices are based on proportions, it does not matter whether we have 10 or 100 individuals (cf. scenarios 11 and 12). The patterns are similar for both indices, and both increase when the individuals become more evenly distributed over species. Some differences between the two indices are worth pointing out, though. Let 0-0-10-50-40 be shorthand for having 0 individuals for species A, 0 for species B, 10 for species C, 50 for species D and 40 for species E.

- 0-0-10-50-40 (Shannon=0.94, Simpson=2.38) and 0-10-10-10-70 (Shannon=0.94, Simpson=1.92), these are scenarios 13 and 16 in Table 1: The Shannon index is the same, whereas the Simpson index is higher for the former, despite the former having fewer species.

- 0-0-0-30-70 (Shannon=0.61, Simpson=1.72) and 0-0-10-10-80 (Shannon=0.64, Simpson=1.52), these are scenarios 6 and 11 in Table 1: The Shannon index is similar, whereas the Simpson index is higher for the former, despite the former having fewer species.

- 0-0-10-10-80 (Shannon=0.64, Simpson=1.52) and 5-5-5-5-80 (Shannon=0.78, Simpson=1.54), these are scenarios 6 and 11 in Table 1: Simpson assigns similar diversity to these two examples, whereas Shannon regards the second combination as more diverse.

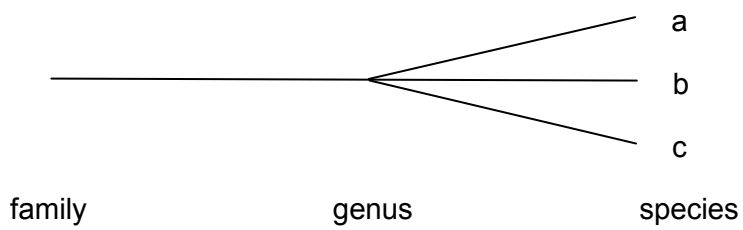
- 0-0-0-50-50 (Shannon=0.69, Simpson=2.00) and 0-0-10-10-70 (Shannon=0.94, Simpson=1.92), these are scenarios 7 and 16 in Table 1: Simpson assigns similar diversity to these two examples, whereas Shannon regards the second combination as more diverse.

Based on these examples it appears that the Simpson index puts more emphasis on the individuals being distributed evenly over species, even if it means fewer species. Nevertheless, for a given number of species both indices show similar behaviour (as can be seen from Table 1 where for a given number of species, both the Shannon and Simpson indices assign increasing diversity to the scenarios presented).

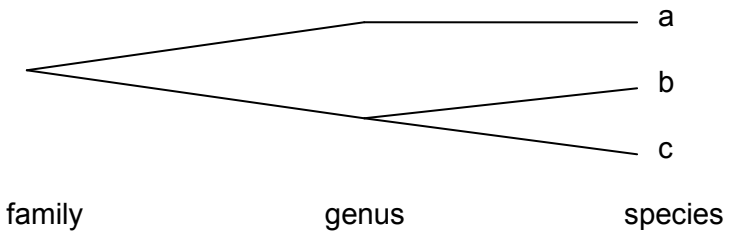
2.1.4 Phylogenetic diversity

The Simpson and Shannon indices assume that all species are equal, i.e. they ignore that species A and B may be part of the same family, whereas species C, D and E may be part of other families (or order, class, phylum, or even kingdom). Phylogenetic diversity indices try to address this issue by regarding species from different families as more diverse than species sharing the same family. The simplest implementation is to draw the phylogenetic tree for a sample of interest, then simplify it so that the highest division (in terms of it occurring at the genus level, family level, order level etc) becomes the top level. This will result in a minimum spanning tree. The phylogenetic diversity is then given by the number of branches in the minimum spanning tree (Faith, 1992). Two illustrations are given below:

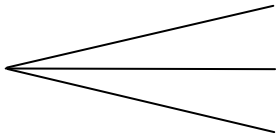
Example 1



Example 2



For the first example, there are no further divisions above the genus level, and the corresponding minimum spanning tree is given as:



This has a phylogenetic diversity of 3. For the second example the phylogenetic tree corresponds to the minimum spanning tree and its phylogenetic diversity is 5 (i.e. 5 branches).

The Shannon and Simpson indices require abundance data, whereas the phylogenetic diversity index only needs incidence data. Unlike the phylogenetic index, the Shannon and Simpson indices do not make use of phylogenetic information.

2.1.5 Taxonomic diversity

Warwick and Clarke (1995) introduced a concept called taxonomic diversity (TD), which is based on the average phylogenetic distance between pairs of species. Let d_{ij} be the phylogenetic distance between species *i* and species *j*, where d=1 if species *i* and *j* belong to the same genus, d=2 if they belong to the same family (but different genera), and so on. In this report we will use the following variations of TD:

- TDsum01: Incidence data, sum of all distances between all possible pairs of species;
- TDavg01: incidence data, the average distance between all possible pairs of species;
- TDsum: abundance data, sum of all distances between all possible pairs of individuals; and

- TDavg: abundance data, the average distance between all possible pairs of individuals (this is the taxonomic diversity introduced by Warwick and Clarke (1995)).

Let x_i and x_j be the total number of individuals belonging to species *i* and *j*, respectively, and let *k* be the total number of species. Then TDsum is defined as:

$$\text{TDsum} = \sum_{\{i=1\}}^{k} \sum_{\{j=1\}}^{\{i-1\}} x_i \, x_j \; d_{ij}$$

This is simply the sum of the distances between all possible pairs of individuals. To obtain TDavg, the need to divide by the total number of pairs:

$$\text{TDavg} = \frac{\sum_{\{i=1\}}^{k} \sum_{\{j=1\}}^{\{i-1\}} x_i \, x_j \; d_{ij}}{\sum_{\{i=1\}}^{k} 0.5 \, x_i(x_i - 1) \; + \; \sum_{\{i=1\}}^{k} \sum_{\{j=1\}}^{\{i-1\}} x_i \, x_j}$$

TDsum01 and TDavg01 are calculated similarly, except that instead of being count of species i, x_i now reflects presence (x_i=1) or absence (x_i=0) of species *i*.

To illustrate, the distance between species a and b in Example 1 in Section 2.1.4 is 1 (they share the same genus), whereas in Example 2 it is equal to 2 (they share the same family). The full calculation of TDsum and TDavg for these Examples is:

Distance between	Example 1	Example 2
(Species a, species b)	1	2
(Species a, species c)	1	2
(Species b, species c)	1	1
TDsum	3	5
TDavg (= TDsum / # species pairs)	1	1.7

2.2 Multidimensional scaling (Principal Coordinates Analysis)

We will use ordination techniques to visualise similarities in species composition between stations. Graphs of the stations, coded according to e.g. sediment-type, median grain size etc, will be created to investigate whether trends in species composition versus sediment type are present.

There are various ways to define the dissimilarity between two stations, all of which are determined by the dissimilarity in their species composition. For incidence data, the Bray Curtis dissimilarity is a popular metric. It is also known as Czekonowski dissimilarity or Sorensen dissimilarity. Let A be the number of species observed at station j, and B be the number of species observed at station k. Let J be the number of species that are found at both stations j and k. Then the Bray Curtis dissimilarity between these two stations is given as (A+B - 2J) / (A+B). In other words, the more species that stations j and k have in common, the lower the dissimilarity. Its minimum value is 0 (the two stations share the same set of species) and its maximum is 1 (the two stations have no species in common). An important feature of this metric is that species which are absent at both stations *j* and *k* do not contribute to the dissimilarity index, and as such it is not influenced by species found at other stations. Other dissimilarity metrics such as Jaccard, Gower, Manhattan, or Canberra are variations of the Bray Curtis metric (i.e. they all have slightly different ways in which A, B, and J are combined into a dissimilarity index). See Legendre & Legendre 1998 for details.

For abundance data (or biomass data), the Bray Curtis dissimilarity between station *j* and station *k* is defined as the sum of the difference in the number of individuals belonging to species *i* between stations *j* and *k*, divided by the total number of individuals (of all species combined) observed at the two stations. In mathematics, the dissimilarity between station *j* and station *k* is given as:

$$\frac{1}{n_j + n_k} \sum_{i=1}^{I} \left| x_{ij} - x_{ik} \right|$$

where x_{ij} and x_{ik} are the total numbers of individuals belonging to species i observed at stations *j* and *k*, respectively, and n_j and n_k are the total numbers of individuals (of all species combined) observed at stations *j* and *k* respectively. For biomass data the same calculation is used (with 'total number of individuals' replaced by 'biomass').

Based on the dissimilarities between stations, graphical displays can be created that try to capture the effect that when two data points (i.e. stations) show a similar species composition they are shown close together in the graphical display. Please note that these graphical displays can be arbitrarily mirrored left to right or top to bottom (this obviously does not change the relative positioning of each of the stations).

Various dissimilarity metrics were explored for the Rock Unique and Solan Bank data, but overall the patterns observed were similar. Therefore, only findings based on the Bray Curtis dissimilarity (for both incidence and abundance data) are presented.

Some issues were encountered with arching in the ordination plots. This is an artefact and is often caused by samples from one end of the arch having few species in common with samples from the other end of the arch (Gauch *et al.* 1977). This may be due to the samples covering a "long ecological gradient" such as going from 0% bedrock to 100% bedrock. For abundance data alternative approaches exist such as the Hellinger transformation but this did not resolve the issue. For practical purposes this means that distances between stations in plots that show arches should be interpreted as distances along the contour of the arch, i.e. stations at opposite ends of the arch are more dissimilar than appears from the graph. Irrespective of the presence or absence of arching, it should be kept in mind that these ordination plots are simplifications of highly complex high-dimensional data, and as such will never form perfect representations of the high-dimensional data.

2.3 α, β and γ diversity

Diversity of the community found at the site can be divided into diversity observed within a station, and diversity observed between stations. The diversity observed within a station is referred to as α diversity, and the diversity of the community of the site as a whole is often referred to as γ diversity. The difference between the two, i.e. the diversity between stations, is the β diversity. These quantities are useful in that they will give an insight into how representative the community observed at a single station is of the community of the entire site.

The various methods outlined in the Methodology Section can be assigned to these forms of diversity as follows:

	Examples
α **diversity**	For data from one station: species richness, Simpson's index, Shannon index, phylogenetic diversity, taxonomic diversity
γ **diversity**	For the combined data from all stations from one habitat: species richness, Simpson's index, Shannon index, phylogenetic diversity, taxonomic diversity
β **diversity**	1 - [Species richness per station/species richness of habitat], 1 - [diversity index per station / diversity index of habitat], Ordination plots from multidimensional scaling

3 Case study: Rock Unique

3.1 Data

Supplemental Figure RU1 shows the locations of the sediment grab sampling stations. Four sediment types were observed, and the number of stations per sediment type is given in Table 2. Twenty-one out of 46 stations had mixed sediment, two stations were classified as mud, whereas the remaining stations were classified as coarse sediment (12 stations) or sand (11 stations).

Table 2. Number of stations per sediment type. Of the 413 entries (rows of data), how many occur per sediment and to what hierarchical level have these been identified. Also shown is the result for the entire site (labelled as 'combined').

			Identified to					
Sediment	**# Stations**	**# Entries**	**Species**	**Genus**	**Family**	**Order**	**Class**	**Phylum**
Coarse	12	234	152	191	217	221	228	234
Mixed	21	318	209	264	295	299	311	318
Mud	2	55	38	46	47	49	52	55
Sand	11	138	89	110	122	125	131	138
Combined	46	413	261	340	385	392	406	413
			As % of Entries					
			Species	**Genus**	**Family**	**Order**	**Class**	**Phylum**
Coarse			65	82	93	94	97	100
Mixed			66	83	93	94	98	100
Mud			69	84	85	89	95	100
Sand			64	80	88	91	95	100
Combined			63	82	93	95	98	100

The Rock Unique data consist of incidence data, abundance data, and biomass data. Results from these data will be presented as follows:

- incidence and abundance data combined, all treated as incidence data;
- abundance data; and
- biomass data.

Sections 3.2-3.4 present the diversity indices, abundances and number of stations required to sample 80% of the total number of species per habitat. This is done for incidence (Section 3.2), abundance (Section 3.3) and biomass data (Section 3.4) separately.

Section 3.5 presents the results from principal coordinates analysis for all three types of data.

3.2 Combined abundance and incidence data (all treated as absence/presence)

Of the 413 entries (i.e. rows in the original data set), about two-thirds were identified to the species level. This went up to 93% at the family level (Table 2). The number of species observed per station was highest for the mixed sediment (37 species per station) and lowest for sand (21 species per station) (Table 3). This pattern was consistent across hierarchical levels.

Table 3. Average number of species, genera etc observed per station, based on incidence data (including abundances treated as incidences) available for each hierarchical level[1].

Sediment	# Stations	Species	Genus	Family	Order	Class	Phylum
coarse	12	32.6[b]	36.3[b]	33.5	22.5	11.3	8.5
mixed	21	37.0[a]	41.5[a]	36.9	23.2	12.1	8.9
mud	2	25.0[c]	29.0[c]	28.0	20.5	12.0	8.5
sand	11	21.1[c]	25.2[c]	24.5	17.5	9.5	7.6
P-value[2]		0.020	0.027	0.066	0.063	0.096	0.22

[1]This explains why the number of species tends to be less than the number of genera as not all data were identified that the species level.

[2]P-value for effect of habitat on diversity per station, based on one-way ANOVA. Means not sharing a superscript are significantly different (P<0.05).

3.2.1 Species accumulation curves

Figure 1 shows species accumulation curves for the four sediment types. For a given number of stations the total number of species identified for sand is only about two-thirds of the total number of species identified for mixed sediment. The number of species observed for the coarse sediment is slightly less than for the mixed sediment.

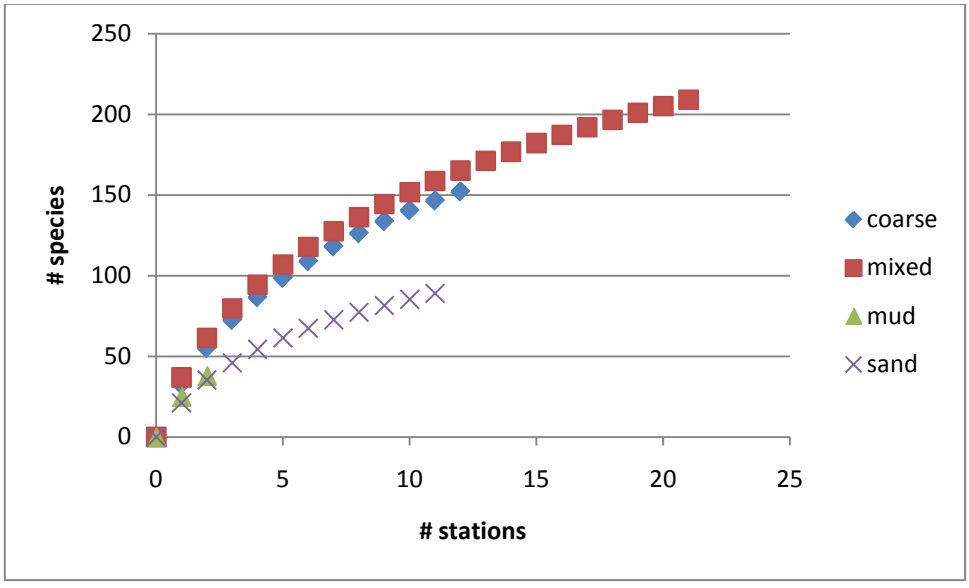


Figure 1. Average number of species observed, per sediment-type, when we have 1, 2, or more stations. Based on incidence data.

3.2.2 Estimated total number of species

The total number of species for each sediment type was estimated using Chao's index, shown in Table 4. This is based on treating all the data as incidence data and therefore the results from the mud sediment, which had only two stations, should be treated with caution (as the Chao index obtained this way is based on the number of stations at which a species is observed). The estimated species richness for sand, coarse sediment and mixed sediment is 146, 216 and 295 species respectively.

Table 4. Observed and estimated (based on Chao) number of species, genera and families per sediment-type. Based on incidence data (including abundances treated as incidences).

	Sediment	Coarse	Mixed	Mud	Sand
	# Stations	12	21	2	11
Species	**Observed**	152	209	38	89
	Estimated	216	295	66	146
Genus	**Observed**	164	210	42	94
	Estimated	213	263	63	156
Family	**Observed**	119	140	39	78
	Estimated	146	178	53	104

3.2.3 Phylogenetic diversity

The phylogenetic diversity, reported in the Table 5, was highest for the mixed sediment (γ diversity) followed by the coarse sediment. To account for differences in the number of stations per sediment (as more stations may result in more species), the phylogenetic diversity was also calculated for each station separately (α diversity). Again, the mixed and coarse sediments show the highest diversity per station. The average diversity per station for sand was significantly less than for the mixed sediment stations ($P<0.05$). Comparing the average diversity per station (i.e. α diversity) to the diversity of the corresponding sediment (i.e. γ diversity), we find that the diversity per sand station was only 29% of that of the sand sediment, indicating a β diversity of 71%. For the coarse and mixed sediments the β diversity was 27 and 23%, respectively. Similar patterns were observed for data identified to at least the family level although there were no longer any statistically significant differences between the sediment types (Supplemental Table RU1).

Table 5. Phylogenetic diversity (PD) for each sediment based on data identified down to the species level. PD was also calculated for each station and then averaged per sediment. Based on incidence data (including abundances treated as incidences).

			per Station	
Sediment	**# Stations**	**Sediment PD γ Diversity**	**Avg PD α Diversity**	**As% of Sediment PD 100 - β diversity**
Coarse	12	461	123[ab]	*27*
Mixed	21	589	138[a]	*23*
Mud	2	144	102[ab]	*71*
Sand	11	285	84[b]	*29*
P-value[1]			0.021	

[1]P-value for effect of sediment on diversity per station, based on one-way ANOVA. Means not sharing a superscript are significantly different (P<0.05).

3.2.4 Number of stations needed per biotope to achieve a given coverage

To sample 80% of all species found per sediment type, 22, 29 and 37 stations would be needed for the coarse sediment, sand, and mixed sediments, respectively (Table 6). If we are only interested in sampling 80% of all families then the number of stations needed is roughly halved, and 12, 15 and 24 stations would be needed for the coarse sediment, sand, and mixed sediment respectively.

Table 6. Number of stations needed per sediment-type to achieve 70 to 95% coverage of the total number of species, genera of families. Based on incidence data (including abundances treated as incidences).

	Sediment % Sampled	**Coarse**	**Mixed**	**Mud**	**Sand**
Species	70	13	22	4	17
	75	17	28	5	22
	80	22	37	6	29
	85	31	53	9	41
	90	50	84	14	65
	95	106	177	29	136
Genus	70	10	14	2	17
	75	13	18	3	22
	80	17	24	4	29
	85	24	35	6	41
	90	38	55	9	65
	95	80	116	20	136
Family	70	7	14	2	9
	75	9	18	2	11
	80	12	24	3	15
	85	18	34	4	22
	90	28	53	7	34
	95	59	112	15	73

3.2.5 Number of stations needed per sediment to detect changes in species richness

We may want to compare the average number of species per station from a mixed sediment from location A with that from a mixed sediment from location B. We may want to compare the average species richness per station from a mixed sediment with that from a sandy sediment, or we may want to compare the average species richness per station from a

mixed sediment observed in 2010 to its average richness five years later. Each of these questions is answered in the same way based on power calculations. The number of stations needed depends on (i) the minimum change in the average richness per station we want to detect, and (ii) variation in species richness between stations. If we are only interested in detecting large changes and if the species richness is similar between stations then obviously only a small number of stations would be required.

Table 7 shows how, if we want to detect a change in species richness per station of 10 or more, 29 stations would be needed. If we want to detect a change in average species richness per station of 4 or more then 173 stations would be needed, which illustrates that to be able to detect a small change a large amount of additional sampling effort is needed. These calculations assume that the sampling effort per station is similar to that employed for the current data (i.e. if for the current data 1 cubic metre of sediment was analysed then the calculations above assume that future samples would also consist of 1 cubic metre of sediment).

Table 7. Number of stations needed per sediment to detect a change of 2, 4, 6,… 20 species (genera, families etc) in the average species richness per station. The between station spread (i.e. standard deviation) in the number of species, genera etc is given in the second column. The numbers in the body of the table are the number of stations needed per sediment. Based on power of 80% at the 5% significance level. Based on incidence data (including abundances treated as incidences).

		Change in number of species, genera etc						
	Spread	**2**	**4**	**6**	**8**	**10**	**15**	**20**
Species	13.2	688	173	78	44	29	14	8
Genus	14.2	786	198	89	51	33	15	9
Family	12.3	590	149	67	38	25	12	7
Order	5.6	127	33	15	9	7	4	3
Class	2.7	29	8	5	4	3	2	2
Phylum	1.5	11	4	3	3	2	2	2

3.3 Abundance data

Out of 413 entries (rows of data), 338 were recorded as counts. Two-thirds of these were identified to the species level, this went up to 94% identified the family level (Table 8). The average number of species per station ranged from 21 for sand, up to 31 for mixed sediment (Table 9).

Table 8. Of the 338 entries (rows of data) that were recorded as abundances, how many occur per sediment and to what hierarchical level have these been identified. Also shown is the result for the entire site (labelled as 'combined').

			Identified to					
Sediment	**# Stations**	**# Entries**	**Species**	**Genus**	**Family**	**Order**	**Class**	**Phylum**
Coarse	12	178	121	148	168	171	176	178
Mixed	21	261	172	217	243	247	258	261
Mud	2	50	36	44	45	47	49	50
Sand	11	125	85	104	114	117	122	125
Combined	46	338	219	280	317	323	335	338
			As % of Entries					
			Species	**Genus**	**Family**	**Order**	**Class**	**Phylum**
Coarse			68	83	94	96	99	100
Mixed			66	83	93	95	99	100
Mud			72	88	90	94	98	100
Sand			68	83	91	94	98	100
Combined			65	83	94	96	99	100

Table 9. Average number of species, genera, etc per station per sediment type, for entries that have been recorded as abundances.

Sediment	**# Stations**	**Species**	**Genus**	**Family**	**Order**	**Class**	**Phylum**
Coarse	12	25.5^{b}	28.5^{b}	25.7	20.1	9.3	6.8
Mixed	21	30.9^{a}	35.1^{a}	30.7	21.0	10.0	7.1
Mud	2	24.0^{bc}	28.0^{b}	27.0	20.0	11.5	8.0
Sand	11	20.6^{c}	24.5^{b}	23.6	17.0	9.0	7.1
P-value[1]		0.023	0.023	0.078	0.17	0.19	0.52

[1]P-value for effect of sediment on diversity per station, based on one-way ANOVA. Means not sharing a superscript are significantly different ($P<0.05$).

3.3.1 Estimated total number of species

The Chao estimates for the total number of species based on abundance data indicate that 57 species are estimated for mud, increasing to 250 for the mixed sediment (Table 10). It is estimated that at least 41 stations would be needed for the mixed sediment to sample 80% of all the species found in this habitat (Table 11).

Table 10. Observed and estimated (based on Chao) number of species, genera and families per sediment-type. Based on abundance data.

	Sediment	Coarse	Mixed	Mud	Sand
	# Stations	12	21	2	11
Species	Observed	121	172	36	85
	Estimated	158	250	57	126
Genus	Observed	127	171	40	88
	Estimated	146	215	64	132
Family	Observed	87	109	37	71
	Estimated	96	131	50	88

Table 11. Number of stations needed per sediment-type to sample 70 to 95% of the total number of species, genera or families. Based on abundance data.

	Sediment % Sampled	Coarse	Mixed	Mud	Sand
Species	70	10	24	3	13
	75	13	30	4	17
	80	18	41	5	22
	85	25	58	7	32
	90	40	91	11	51
	95	84	193	23	107
Genus	70	6	14	3	13
	75	8	18	4	17
	80	11	25	5	22
	85	15	35	7	31
	90	25	55	11	50
	95	52	116	23	105
Family	70	5	11	2	7
	75	6	14	2	9
	80	8	19	3	11
	85	11	27	4	16
	90	18	43	7	26
	95	37	90	14	54

The average estimated total number of species per station ranges from 20 to 68, so that the average observed number of species per station amounts to 44 to 61% of the estimated total number of species per station (Table 12).

Table 12. Observed (obs) and estimated (est) number of species per sediment, and the average per station per sediment. Based on abundance data.

	Per Sediment			Avg per Station			
	Est	Obs	Obs as % of Est	Est	Est as % of sediment Est	Obs	Obs as % of Est per station
Coarse	158	121	76	51	*32*	26	50
Mixed	250	172	69	51	*20*	31	61
Mud	57	36	63	39	*68*	24	61
Sand	126	85	67	47	*37*	21	44

3.3.2 Diversity

As we are now dealing with abundance data, in addition to phylogenetic diversity we can also look at Shannon and Simpson diversity indices (Table 13). All three indices indicate that sand shows the lowest average diversity per station (i.e. α diversity), but this is only statistically significant for the phylogenetic diversity index. The phylogenetic diversity per station is 24 to 30% of that of the corresponding sediment (sand, coarse and mixed sediments), suggesting a β diversity of 70 to 76%. When looking at data identified to the family stratum we find no differences between the sediments (Supplemental Table RU2). Beta diversity ranges from 58-64% for sand, coarse and mixed sediments.

Table 13. Diversity for each sediment based on abundance data identified down to the species level. Diversity was also calculated for each station and then averaged per sediment.

		Total Diversity per Sediment (γ diversity)			Average per Station (α diversity)			Station Avg as % of Total (100 - β diversity)		
Sediment	**# Stations**	**Shannon**	**Simpson**	**PD**	**Shannon**	**Simpson**	**PD**	**Shannon**	**Simpson**	**PD**
Coarse	12	3.94	28.2	374	2.81	12.7	99^{ab}	*71*	*45*	*27*
Mixed	21	3.97	26.9	485	2.93	13.0	117^{a}	*74*	*48*	*24*
Mud	2	3.25	20.0	135	2.94	15.0	98^{ab}	*91*	*75*	*72*
Sand	11	3.88	31.3	269	2.74	12.3	82^{b}	*71*	*39*	*30*
P-value[1]					0.32	0.84	0.037			

[1]P-value for effect of sediment on diversity per station, based on one-way ANOVA. Means not sharing a superscript are significantly different (P<0.05).

Taxonomic diversity, calculated as the average path length between species pairs (TD01avg) or between individual pairs (TDavg), is shown in Table 14 (for data identified to the species level) and Supplemental Table RU3 (for data identified to the family level). Both at the species and family level, mud shows the highest taxonomic diversity per station (albeit that the differences between the sediments are statistically significant at the family level only). Unlike phylogenetic diversity, it is possible for the average distance between species pairs or individual pairs to be bigger for an individual station than for the sediment as a whole (this is the case for mud at the family level).

Table 14. Taxonomic diversity for each sediment based on abundance data identified down to the species level. Taxonomic diversity was also calculated for each station and then averaged per sediment. Taxonomic diversity was calculated as the average distance between all possible pairs of individuals (TDavg) or pairs of species (TD01avg).

		Diversity per Sediment		Average per Atation	
Sediment	**# Stations**	**TDavg**	**TD01avg**	**TDavg**	**TD01avg**
Coarse	12	5.08	5.27	4.83	5.14
Mixed	21	5.10	5.26	4.75	5.08
Mud	2	5.11	5.27	5.10	5.31
Sand	11	5.04	5.13	4.91	5.11
P-value				0.174	0.519

3.3.3 Comparison of diversity indices

Supplemental Figures RU2 (species level) and RU3 (family level) show the Simpson, Shannon, phylogenetic and taxonomic diversity indices for each station (i.e. α diversity). The indices are coloured from green through to red to reflect low to high values. Species richness, phylogenetic diversity and the sum of the distances between individual pairs (TDsum) or species pairs (TD01sum) show agreement in which stations are diverse and which stations are not. The Simpson and Shannon indices show similar patterns. An exception is station 185, which shows a high phylogenetic diversity index and a low Simpson (and Shannon) index. This station has a large number of species, but because counts are high for some of the species (including counts of 10, 22, and 37 for some species) and not for others (counts of 1 or 2) this suppresses the Simpson (and Shannon) index as the total individual count is not evenly distributed among species. On the other hand, station 217 shows a moderate phylogenetic diversity index and a high Simpson (and Shannon) index. This is because the total individual count is rather evenly distributed among the species (with individual species having counts ranging from 1 to 6). When taxonomic diversity is expressed as the average path length between individual pairs (TDavg) or species pairs (TD01avg) the pattern is somewhat different. This is a reflection of the fact that, unlike phylogenetic diversity and TDsum, the average path length does not contain any information about species richness.

Figure 2 shows the various diversity indices plotted against each other. Phylogenetic diversity (PD), taxonomic diversity in terms of sum of distances between individual pairs (TDsum) or species pairs (TD01sum), and Shannon diversity all show a strong relationship with species richness (i.e. the total number of species). Simpson's index relates to the Shannon index. Notably, the average distance between species pairs (TD01avg) or individual pairs (TDavg) does not relate to the number of species as these two indices do not contain information on species richness.

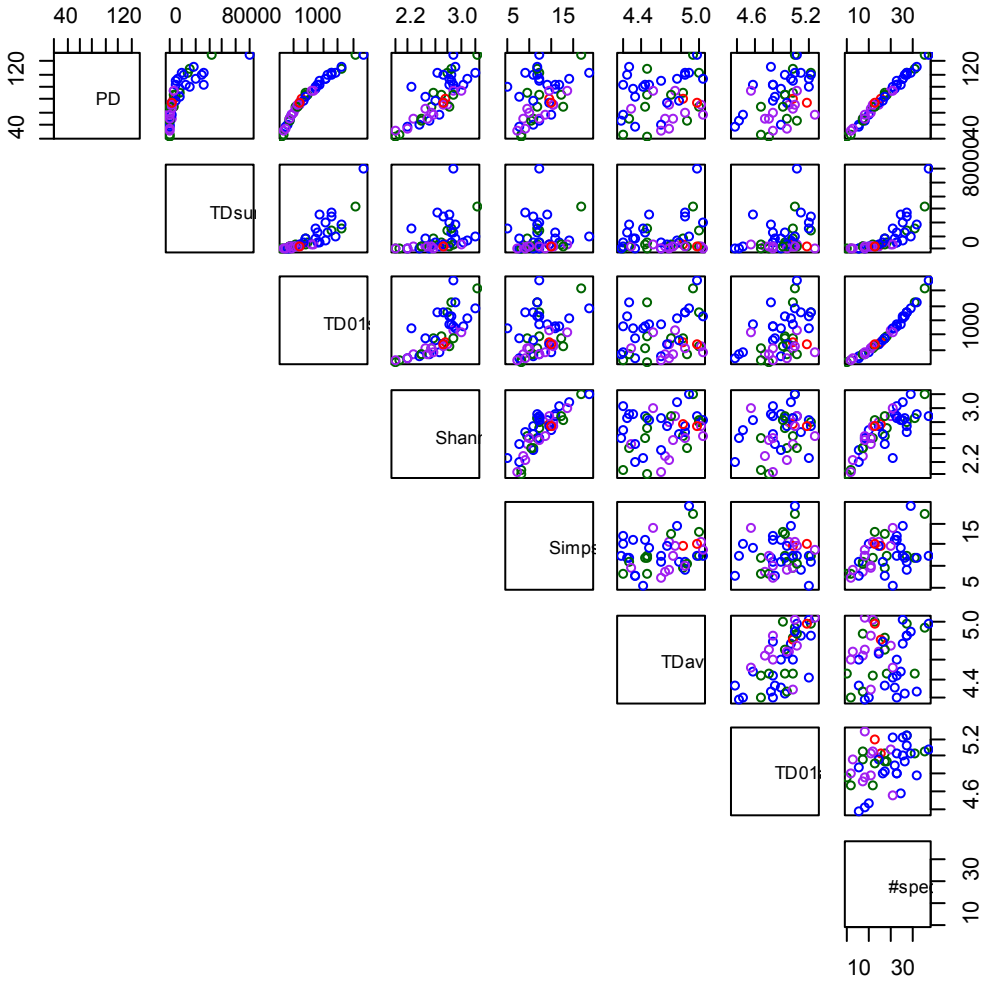


Figure 2. Comparing Shannon, Simpson, phylogenetic diversity (PD) and taxonomic diversity indices. (TDsum: sum of distances between pairs of individuals or pairs of species (TD01sum); TDavg: average distance between pairs of individuals or pairs of species (TD01avg)), calculated for each station using species abundance data. Also shown is the number of species. Stations are coloured according to their sediment type (red= mud, green = coarse sediment, blue = mixed sediment, purple = sand).

Table 15 shows the correlations between diversity indices and sediment characteristics. To avoid undue influence of outliers, nonparametric correlations (i.e. Spearman rank correlation) were used. The Shannon and Simpson indices did not correlate with sediment characteristics. The percentage mud did not correlate with any of the diversity indices. Species richness, phylogenetic diversity, and taxonomic diversity (sum of all pairwise phylogenetic distances, TDsum) showed a positive association with the percentage gravel and a negative association with sand, which is not surprising as sand and gravel show a strong negative association. The average taxonomic diversity (TDavg) shows a positive association with skewness and kurtosis, and the negative association with sorting. Figures 3 and 4 show the relationship for a species richness, Shannon index, phylogenetic diversity and taxonomic diversity. It is worth noting that, although some of the relationships are statistically significant, in practical terms the associations are not strong (a correlation of 0.33 means that only 0.33^2 =10% of the variation in Y is explained by X). The strongest correlation observed was -0.49, i.e. no more than 24% of the variation in diversity indices is explained by sediment characteristics.

Table 15. Correlations (non-parametric Spearman correlation) and corresponding P-values between various measures of diversity and sediment characteristics (based on log φ particle size determination). Significant correlations are shown in bold.

	Spearman correlation							
	Mean	**Sorting**	**Skewness**	**Kurtosis**	**D50**	**Gravel%**	**Sand%**	**Mud%**
#species	**-0.34**	**0.30**	-0.02	-0.26	**-0.38**	**0.45**	**-0.49**	-0.03
PD	**-0.34**	0.27	0.00	-0.24	**-0.38**	**0.44**	**-0.48**	-0.05
TDsum	**-0.37**	**0.32**	-0.05	**-0.30**	**-0.41**	**0.47**	**-0.53**	-0.04
TDavg	0.22	**-0.42**	**0.35**	**0.43**	0.10	-0.25	0.27	0.08
Shannon	-0.15	0.12	0.10	-0.06	-0.21	0.23	-0.27	0.05
Simpson	0.04	-0.06	0.21	0.11	-0.03	-0.01	-0.06	0.14
	P-values							
	Mean	**Sorting**	**Skewness**	**Kurtosis**	**D50**	**Gravel**	**Sand**	**Mud**
#species	0.019	0.046	0.896	0.086	0.009	0.002	0.001	0.856
PD	0.019	0.071	0.984	0.114	0.009	0.002	0.001	0.737
TDsum	0.011	0.029	0.744	0.040	0.005	0.001	0.000	0.788
TDavg	0.151	0.004	0.017	0.003	0.508	0.090	0.074	0.580
Shannon	0.304	0.431	0.520	0.678	0.164	0.124	0.068	0.750
Simpson	0.785	0.709	0.151	0.460	0.855	0.962	0.709	0.357

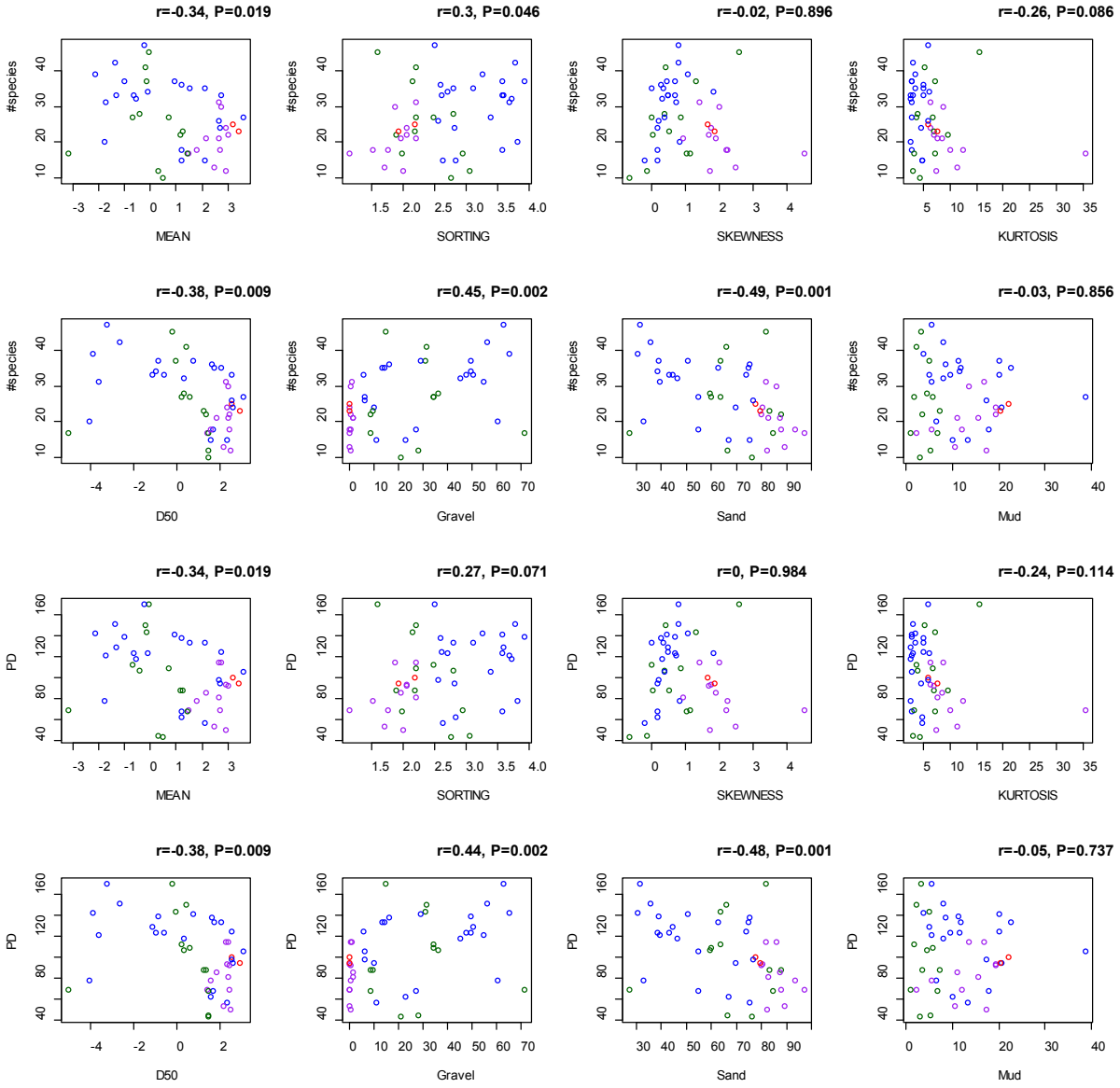


Figure 3. Relationship between species richness (top two rows) and sediment characteristics, and phylogenetic diversity and sediment characteristics (bottom two rows). Based on species abundance data. Stations are coloured according to their sediment type (red= mud, green = coarse sediment, blue = mixed sediment, purple = sand). Spearman rank correlation (r) and its P-value (P) are shown in figure headers. Sediment characteristics based on log ϕ particle size. Gravel, sand and mud are expressed as % of total sediment composition.

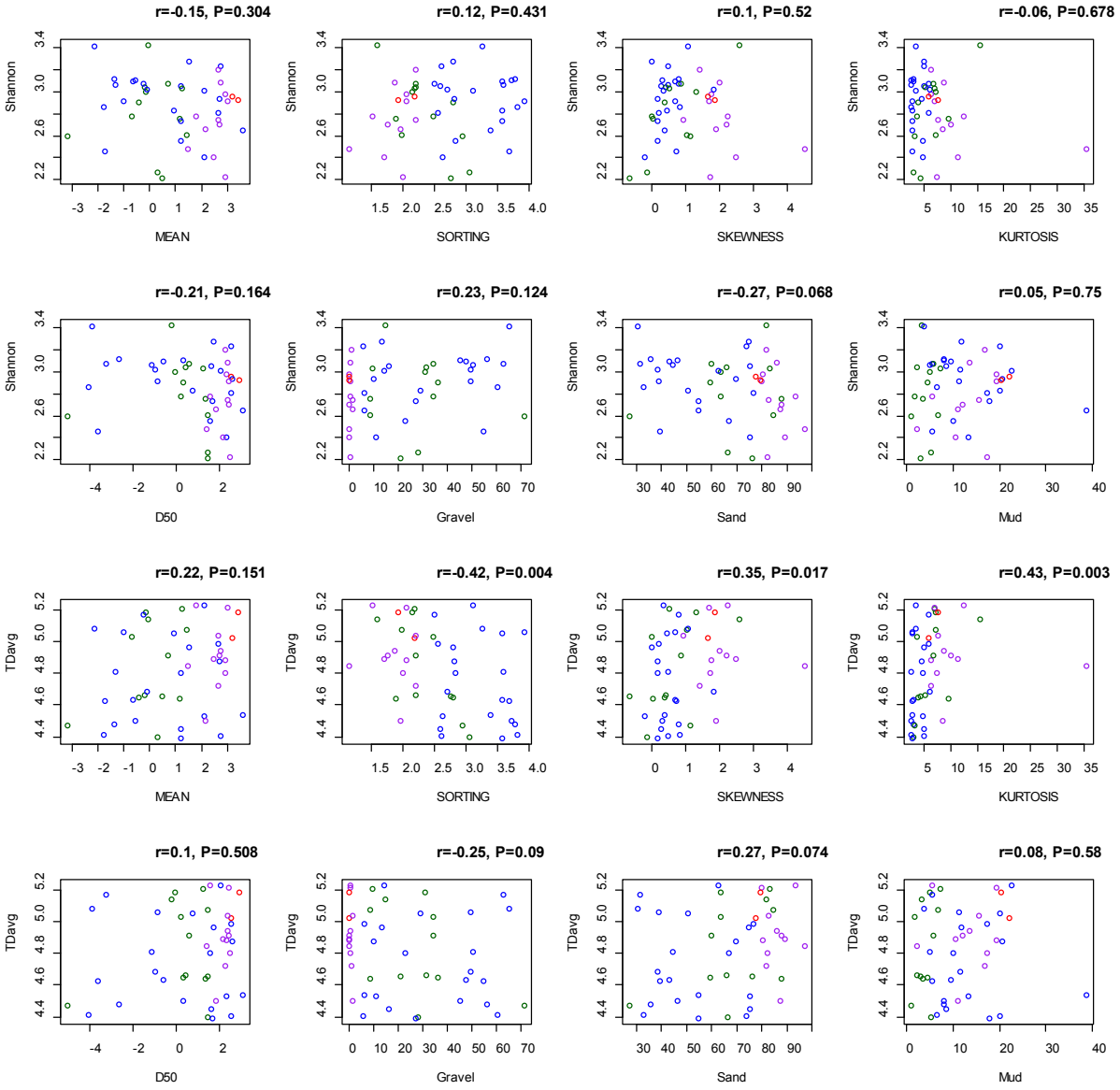


Figure 4. Relationship between Shannon index (top two rows) and sediment characteristics, and taxonomic diversity (average path length between any pair of individuals) and sediment characteristics (bottom two rows). Based on species abundance data. Stations are coloured according to their sediment type (red= mud, green = coarse sediment, blue = mixed sediment, purple = sand). Spearman rank correlation (r) and its P-value (P) are shown in figure headers. Sediment characteristics based on log ϕ particle size. Gravel, sand and mud are expressed as % of total sediment composition.

3.4 Biomass data

For 306 entries (rows in data matrix) biomass data are available. Two-thirds of these were identified to the species level, this went up to approximately 90% identified at the family level (Table 16). The average number of species per station was highest for the mixed sediments (Table 17), and this sediment also had the highest biomass (Table 18).

Table 16. Of the 306 entries (rows of data) that were recorded as biomass, how many occur per sediment and to what hierarchical level have these been identified. Also shown is the result for the entire site (labelled as 'combined').

			Identified to					
Sediment	**# Stations**	**# Entries**	**Species**	**Genus**	**Family**	**Order**	**Class**	**Phylum**
Coarse	12	167	112	137	153	156	162	167
Mixed	21	235	161	200	218	220	229	235
Mud	2	50	35	42	42	44	47	50
Sand	11	122	80	97	107	110	116	122
Combined	46	306	204	258	286	290	300	306
			As % of Entries					
			Species	**Genus**	**Family**	**Order**	**Class**	**Phylum**
Coarse			67	82	92	93	97	100
Mixed			69	85	93	94	97	100
Mud			70	84	84	88	94	100
Sand			66	80	88	90	95	100
Combined			67	84	93	95	98	100

Table 17. Average number of species, genera etc observed per station, based on biomass data available for each hierarchical level.

Sediment	**# Stations**	**Species**	**Genus**	**Family**	**Order**	**Class**	**Phylum**
Coarse	12	21.6^{b}	24.2^{b}	21.9	18.2	7.8	6.3
Mixed	21	27.6^{a}	31.0^{a}	27.3	19.4	8.9	6.6
Mud	2	23.0^{b}	25.0^{b}	25.0	17.0	11.0	7.0
Sand	11	19.1^{b}	22.5^{b}	22.2	15.9	8.5	6.7
P-value[1]		0.019	0.025	0.079	0.20	0.13	0.77

[1]P-value for effect of sediment on diversity per station, based on one-way ANOVA. Means not sharing a superscript are significantly different (P<0.05).

Table 18. Average biomass per station, based on biomass data available for each hierarchical level.

Sediment	**# Stations**	**Species**	**Genus**	**Family**	**Order**	**Class**	**Phylum**
Coarse	12	0.96	1.11	1.13	1.34	1.51	2.11
Mixed	21	3.45	3.70	3.72	3.72	3.87	3.97
Mud	2	0.66	0.69	0.69	0.88	1.06	1.10
Sand	11	0.80	1.10	1.12	1.12	1.21	1.34
P-value[1]		0.16	0.14	0.15	0.18	0.17	0.24

[1]P-value for effect of sediment on diversity per station, based on one-way ANOVA.

3.4.1 Diversity

Table 19 shows the diversity indices based on biomass data. No differences in diversity observed based on Shannon and Simpson indices, whereas for the phylogenetic diversity index the mixed sediment shows the highest α diversity. Looking at data identified at the family level we do not observe any differences in diversity between sediment types (Supplemental Table RU4). Beta diversity is similar to that observed for the incidence and abundance data, ranging from 70-77% for species data and 60-67% for the family data.

Table 19. Diversity for each sediment based on biomass data identified down to the species level. Diversity was also calculated for each station and then averaged per sediment.

		Total Diversity per Sediment (γ Diversity)			Average per Station (α Diversity)			Station Avg as % of Total (100 - β Diversity)		
Sediment	**# Stations**	**Shannon**	**Simpson**	**PD**	**Shannon**	**Simpson**	**PD**	**Shannon**	**Simpson**	**PD**
Coarse	12	2.68	6.88	349	1.51	3.55	86[b]	*56*	*52*	*25*
Mixed	21	2.47	6.49	451	1.67	4.08	106[a]	*67*	*63*	*23*
Mud	2	2.47	7.21	132	2.03	5.83	93[ab]	*82*	*81*	*70*
Sand	11	2.94	8.38	255	1.79	4.67	76[b]	*61*	*56*	*30*
P-value[1]					0.64	0.54	0.028			

[1]P-value for effect of sediment on diversity per station, based on one-way ANOVA. Means not sharing a superscript are significantly different (P<0.05).

3.5 Principal coordinates analysis

Figure 5 gives a visual representation of β diversity, based on results from principal coordinates analysis applied to all available incidence data at the species level, genus level, family level etc. The percentages along the x-axis and y-axis give an indication of how well the graphs represent the actual dissimilarities. The observed pattern is similar for each of the hierarchy levels. Please note that top and bottom can be arbitrarily mirrored and likewise left and right can be arbitrarily mirrored (the graph for incidence data identified to at least the family level has top and bottom reversed compared to the species and genus graphs). The arch-shape is an artefact and the distances between stations should be 'read' along the arch. In other words, stations at either end of the arch are more different than appears from the graph.

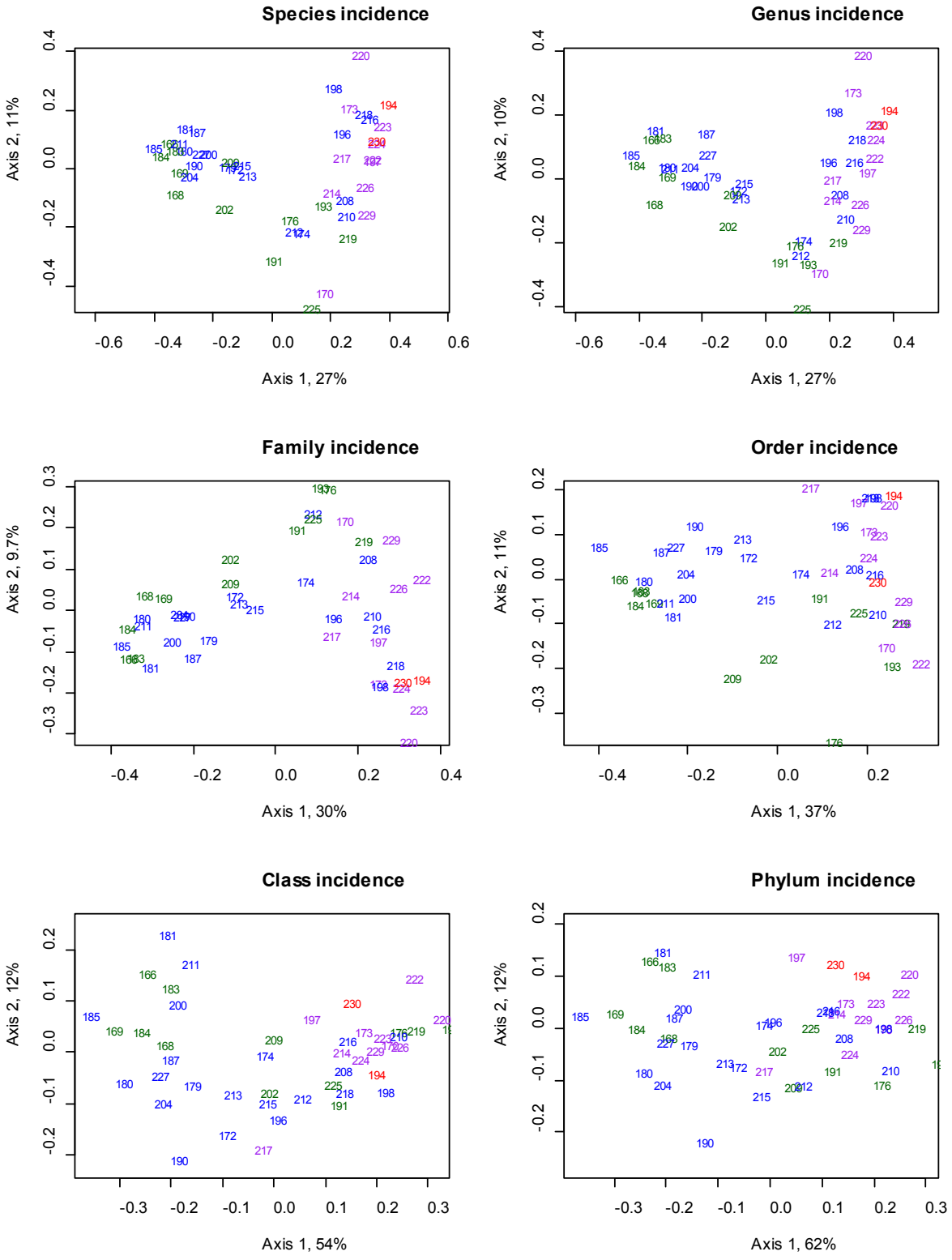


Figure 5. PCO analysis using incidence data (including abundances treated as incidences) identified to species level, genus level etc. Stations are reflected by their codes and are coloured according to their sediment type (red= mud, green = coarse sediment, blue = mixed sediment, purple = sand).

Supplemental Figure RU4 shows the results from PCO applied to abundance data, and the patterns are similar to those from the incidence data (Figure 5). For the biomass data, the graphs look somewhat different (Supplemental Figure RU5), but note that these do not reflect the dissimilarities between stations quite as well as the graphs shown in Figure 5 and Supplemental Figure RU4 (this can be seen from lower % on the axes). Closer inspection reveals however that similar dissimilarities between stations are observed (i.e. stations that are close together based on incidence or abundance data also tend to be close together for the biomass data).

The three sets of results show that stations tend to cluster according to sediment-type. The two mud stations (shown in red) tend to appear close together, and the sand (purple) stations tend to group together.

To investigate in more detail which of the station characteristics might be responsible for these patterns, we look at the data identified to the species level and to the family level in more detail. The reason for choosing the family level is that approximately 90% of the entries have been identified to this level, whilst at the same time being sufficiently low down the hierarchy tree to still contain some detail in terms of diversity. Figure 6 shows, for the incidence data analysed at the species level, what the characteristics of the stations are. Each sub-plot shows the same configuration of stations, but the sub-plots differ in how the stations have been indicated by circles. For example, the top left graph shows the percentage of gravel found at each station, where larger circles indicate a larger contribution. From these graphs it follows that stations appearing towards the left of the configuration are associated with a higher gravel content, lower sand content, higher phylogenetic diversity, and lower kurtosis and skewness for particle numbers. Supplemental Figure RU6 shows the same approach for the data classified to at least the family they belong to. The patterns and association with station characteristics are similar to those observed for the species level data.

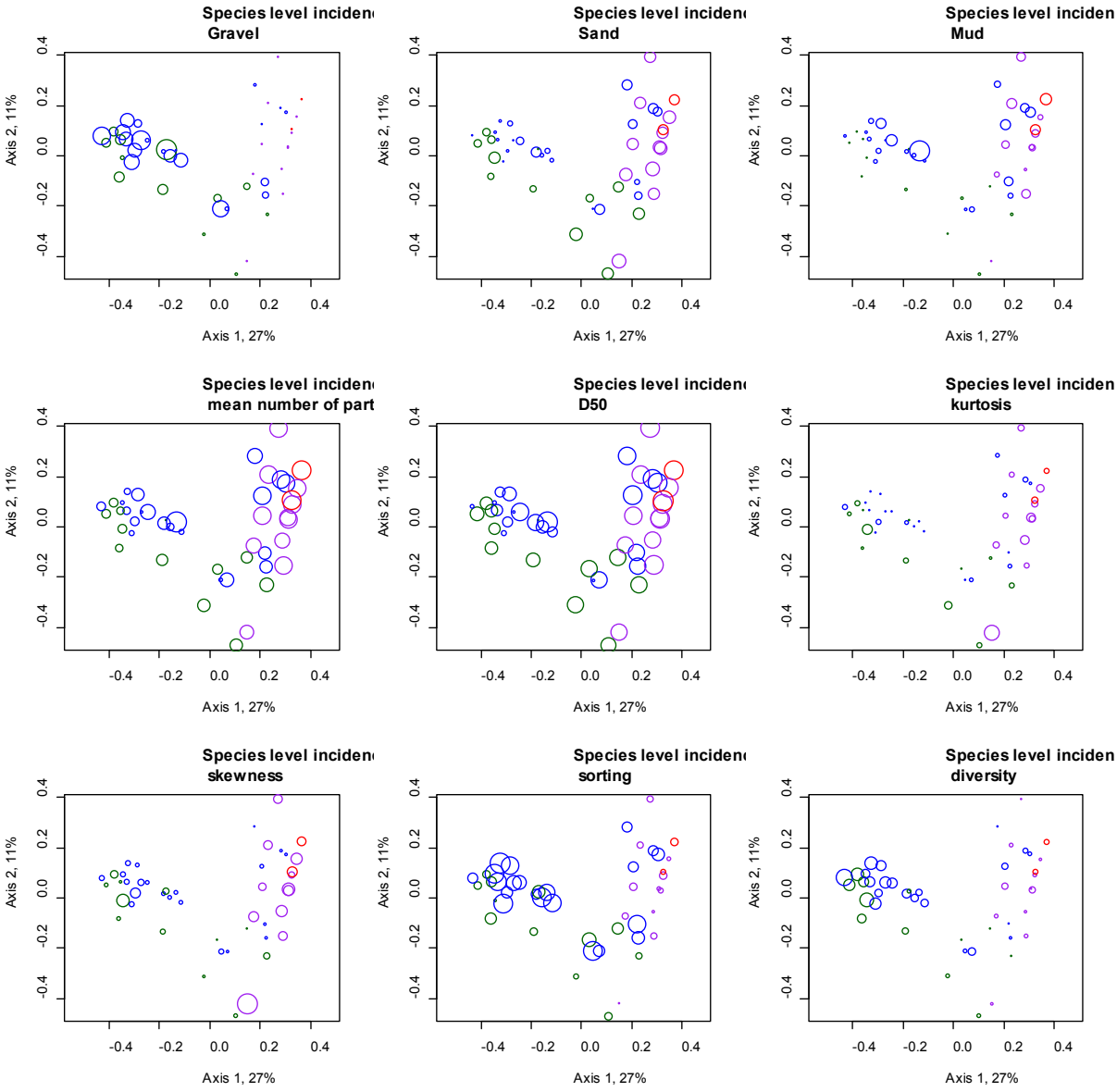


Figure 6. PCO analysis using incidence data (including abundances treated as incidences) identified to species level. Stations are reflected by circles, where the diameter size reflects percentage of gravel, sand, mud (first row), mean number of particles, median number of particles, kurtosis, skewness or sorting (latter five indices based on log ϕ particle size determination). The final plot shows phylogenetic diversity. The colours reflect sediment type (red= mud, green = coarse sediment, blue = mixed sediment, purple = sand).

Supplemental Figures RU7 and RU8 show similar patterns for the corresponding abundance data. Gravel and sand appear to be the best predictors for species composition and this agrees with their correlation with the various diversity indices being strongest (Table 15). For the biomass data any patterns are less clear (Supplemental Figures RU9 and RU10).

Are stations that are geographically close or share the same sediment more similar than stations further apart?

Figures 5 and 6 and Supplemental Figures RU4-RU10, in combination with Supplemental Figure RU1, show that there are stations that are geographically close are also similar in species composition. Examples are stations 184, 185 (despite having different sediments, namely a coarse sediment for station 184 consisting of 31% gravel, 66% sand and 2% mud,

and a mixed sediment for station 185 consisting of 62% gravel, 31% and 5% mud), and stations 217, 218 (despite having different sediments), and stations 196, 197 (despite having different sediments). These latter two examples may be explained by the fact that although the sediments fall into different EUNIS classifications, the gravel/sand/mud composition is similar (i.e. they fall close to the boundary between sediment classes). There are also stations, despite being geographically close, for which the species composition is not all that similar, e.g. 215, 219, and 226, 227 (again, both examples show differences in the EUNIS sediment classification as well as in their gravel/sand/mud composition so perhaps less surprising that these stations are less similar in their species composition). Stations 202, 193 are geographically close (and share the same sediment, albeit that the gravel/sand/mud composition is not identical, namely 34/60/6% for 202 and 20/76/3% for 193), but in terms of species composition less so. Generally, however, Figure 5 and Supplemental Figure RU4 suggest that when stations are in close proximity and share the same sediment, then their species composition tends to be similar. Stations that share the same sediment but are geographically far apart tend to share species, e.g. stations 217 and 224, or stations 196 and 208, or stations 170 and 226. As always, counterexamples can also be found, and stations that are geographically far apart but share the same sediment do not necessarily have a similar species composition, see for example stations 166 and 225.

For the biomass data (Supplemental Figure RU5) patterns are less clear, but overall, when stations are geographically close and share the same sediment then their biomass composition tends to be similar.

3.6 Conclusions

- Two-thirds of the data were identified to the species level. This went up to 93% identified to at least the family level.

- Approximately 57 to 77% of the estimated total species richness was observed.

- To sample 80% of the total number of species per sediment we would need 22, 29 and 37 stations for coarse sediment, sand, and mixed sediment, respectively. To sample 80% of the total number of families per sediment, not quite as many stations would be required, namely 12, 15 and 24 stations for coarse sediment, sand, and mixed sediment, respectively. This is based on treating the data as incidence data but similar numbers were obtained for abundance data. Only two stations were classified as having a muddy sediment and it was not possible to reliably estimate the number of stations required.

- To investigate diversity, species richness, Shannon's index, Simpson's index and two measures of phylogenetic complexity were investigated. Phylogenetic diversity measures the complexity of the phylogenetic tree by counting the total number of branches, whereas taxonomic diversity measures this complexity by looking at the total number of branches that separate pairs of species from one another. Two measures for taxonomic diversity were used; the sum of all path lengths between all possible species pairs, and the average path length between all possible species pairs.

    - Phylogenetic diversity and taxonomic diversity (sum of path lengths), and to a lesser extent Shannon diversity, all showed a strong association with species richness.

    - Phylogenetic diversity, taxonomic diversity (sum of path lengths) Simpson, Shannon, and species richness showed similar findings in that they tend to agree on which stations are most (or least) diverse.

- The strongest associations between sediment type and diversity were observed with sand and gravel, where diversity showed a negative association with the % sand and a positive association with the % gravel. Despite the statistical significance of these relationships, in practice less than 25% of the variation in diversity was explained by the % sand or % gravel.

- Ordination techniques showed similar patterns for incidence and abundance data. Patterns were also similar looking at data identified to the species level, genus level or family level (despite the family level containing more data). The stations tend to cluster according to sediment type, mainly sand and gravel. Stations that share the same sediment and are in close proximity geographically tend to cluster together in the ordination plots.

4 Case study: Solan Bank

4.1 Data structure

The data structure is as follows. There are 24 video stations, where data from each 'station' were obtained from a video tow. Several stills were obtained per tow (or station). These stills cover one or more biotopes. To illustrate:

Station	Biotope	Stills code	Species[1]
Station 45	CR.MCR.EcCr.CarSp.Bri	DC13_02 DC13_04 DC13_05 DC13_06	A,B,C B,C,D,E A,B,E,F F,G
Station 45	SS.SCS.OCS	DC13_01 DC13_03 DC13_07	A,I,J J,K,L,M A,J,M

[1]Acts as illustration

The data were then restructured in two ways, namely:

- summaries of species observed per station per biotope per still, i.e. the species observed for an individual still; and
- Summaries of species observed per station per biotope, i.e. the species observed in stills 2, 4, 5 and 6 were combined, and the species in stills 1, 3 and 7 were combined. The above data is then given as:

Station	Biotope	Stills code	Species
Station 45	CR.MCR.EcCr.CarSp.Bri	-	A,B,C,D,E,F,G
Station 45	SS.SCS.OCS	-	A,I,J,K,L,M

To avoid the lengthy wording 'per station per biotope' we will use the term 'substation' instead. So Station 45 with biotope CR.MCR.EcCr.CarSp.Bri forms one substation and Station 45 with biotope SS.SCS.OCS forms another substation. Biotopes encountered multiple times within the same tow were aggregated into one biotope (in the example above this might have been the case for stills 1, 3 versus still 7).

4.2 Data

Station 52 was removed as it contained only one observation. Supplemental Figure SB1 shows the locations of the 23 remaining stations. Three of these cover only one biotope. Fifteen stations contain two biotopes each, four stations contain three biotopes each, and one station covers four biotopes. See also Figure 7. As the diversity within a biotope is the main outcome of interest, each station was divided into substations according to biotope. To illustrate, station 92 contains data from four biotopes, so was separated into four substations. This results in 49 substations in total. For each substation, one or more photographs (referred to as stills) were analysed for presence of species.

For simplicity, biotopes will be referred to as biotopes 1 to 8 (Table 20). Of the eight biotopes observed, three biotopes were found at one location only (biotope 2 was observed at station 73, biotope 5 was observed at station 98 and biotope 8 was observed at station 53). Biotopes 1 and 7 were observed in four locations each. The remaining three biotopes, namely biotopes 3, 4 and 6, were observed at 13, 11 and 14 stations (See Table 20 and Figure 7), and the total number of stills for each of these biotopes was 49, 39 and 29, respectively. On average, 2.8 stills were obtained per substation (i.e. per biotope per station).

Table 20. Number of substations (i.e. stations per biotope) and stills per biotope.

Biotope	Code	# Substations	# Stills	Stills/Substation
CR.HCR.FaT.CTub.Adig	1	4	8	2
CR.HCR.XFa.SpAnVt	2	1	2	2
CR.MCR.EcCr.CarSp.Bri	3	13	49	3.77
CR.MCR.EcCr.CarSp.PenPcom	4	11	39	3.55
IR.HIR.KFaR.LhypR.Pk	5	1	1	1
SS.SCS.OCS	6	14	29	2.07
SS.SMx.OMx	7	4	7	1.75
SS.SSa.OSa	8	1	2	2
Total		49	137	2.80

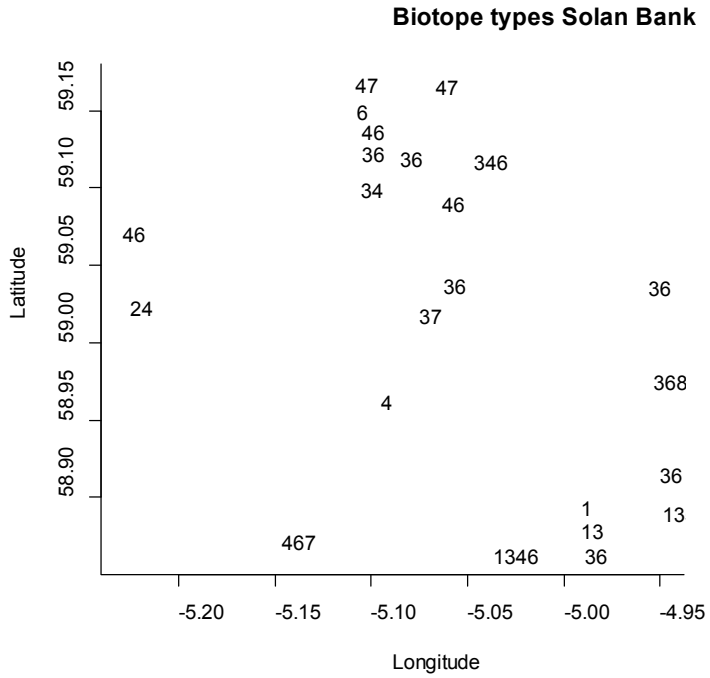


Figure 7. Biotope types found at each station. For example, 37 means that biotopes 3 and 7 were observed at this station. 1= CR.HCR.FaT.CTub.Adig, 2= CR.HCR.XFa.SpAnVt, 3= CR.MCR.EcCr.CarSp.Bri, 4= CR.MCR.EcCr.CarSp.PenPcom, 5= IR.HIR.KFaR.LhypR.Pk, 6= SS.SCS.OCS, 7= SS.SMx.OMx, 8= SS.SSa.OSa.

Of the nearly 3100 observations, 77% were identified down to the species level, this went up to 91% at the family level (Table 21). A total of 103 species were observed (Table 22). Biotope 4 represented 87 of those. Biotopes 8 and 5, which were found at only one station, covered 5 and 17 species each. On the other hand, biotope 2, which was also represented by only one station, covered 45 species. When looking at the average number of species per substation, biotopes 2 and 4 are highest with 45 and 41 species, respectively, followed by biotope 3 with 30 species (Table 23).

Table 21. Numbers of observations (n) per biotope, identified down to species, genus,... phylum level.

Biotope	Code	n	Identified to Species	Genus	Family	Order	Class	Phylum
CR.HCR.FaT.CTub.Adig	1	191	148	158	172	172	174	191
CR.HCR.XFa.SpAnVt	2	84	67	77	80	80	81	84
CR.MCR.EcCr.CarSp.Bri	3	1097	856	924	1000	1001	1027	1097
CR.MCR.EcCr.CarSp.PenPcom	4	1301	985	1109	1190	1193	1229	1301
IR.HIR.KFaR.LhypR.Pk	5	23	17	19	20	20	22	23
SS.SCS.OCS	6	292	220	233	254	254	267	292
SS.SMx.OMx	7	99	70	79	87	87	92	99
SS.SSa.OSa	8	7	7	7	7	7	7	7
Total		3094	2370	2606	2810	2814	2899	3094
			As % of Observations					
			Species	Genus	Family	Order	Class	Phylum
CR.HCR.FaT.CTub.Adig	1		77	83	90	90	91	100
CR.HCR.XFa.SpAnVt	2		80	92	95	95	96	100
CR.MCR.EcCr.CarSp.Bri	3		78	84	91	91	94	100
CR.MCR.EcCr.CarSp.PenPcom	4		76	85	91	92	94	100
IR.HIR.KFaR.LhypR.Pk	5		74	83	87	87	96	100
SS.SCS.OCS	6		75	80	87	87	91	100
SS.SMx.OMx	7		71	80	88	88	93	100
SS.SSa.OSa	8		100	100	100	100	100	100
Total			77	84	91	91	94	100

Table 22. Observed number of species, genera etc per biotope. This based on the total number of available data at each level of the tree[1].

Biotope	Code	# Sub Stations	Number of Species	Genera	Families	Orders	Classes	Phyla	Kingdoms
CR.HCR.FaT.CTub.Adig	1	4	41	44	43	28	16	11	3
CR.HCR.XFa.SpAnVt	2	1	45	47	45	24	14	9	2
CR.MCR.EcCr.CarSp.Bri	3	13	70	74	64	33	17	10	2
CR.MCR.EcCr.CarSp.PenPcom	4	11	87	89	75	36	17	10	2
IR.HIR.KFaR.LhypR.Pk	5	1	17	19	18	17	12	9	3
SS.SCS.OCS	6	14	53	52	51	30	16	10	2
SS.SMx.OMx	7	4	37	41	40	26	16	9	1
SS.SSa.OSa	8	1	5	5	5	4	3	2	1
Total		49	103	105	88	42	19	12	3

[1]This explains why the number of species tends to be less than the number of genera as not all data were identified that the species level.

Table 23. Average number of species, genera etc per substation (i.e. per station per biotope).

Biotope	Code	# Sub Stations	Average number per substation Species	Genera	Families	Orders	Classes	Phyla	Kingdoms
CR.HCR.FaT.CTub.Adig	1	4	21.8	23.0	23.3	17.5	12.0	8.5	2.3
CR.HCR.XFa.SpAnVt	2	1	45.0	47.0	45.0	24.0	14.0	9.0	2.0
CR.MCR.EcCr.CarSp.Bri	3	13	29.5	31.5	31.1	19.4	13.1	8.6	1.9
CR.MCR.EcCr.CarSp.PenPcom	4	11	40.6	44.5	41.1	23.9	14.4	8.7	1.5
IR.HIR.KFaR.LhypR.Pk	5	1	17.0	19.0	18.0	17.0	12.0	9.0	3.0
SS.SCS.OCS	6	14	11.6	12.1	12.2	9.4	7.3	5.6	1.3
SS.SMx.OMx	7	4	14.8	16.5	16.8	11.3	9.5	6.5	1.0
SS.SSa.OSa	8	1	5.0	5.0	5.0	4.0	3.0	2.0	1.0

For the three most prevalent biotopes, the average number of species observed per still is less than two-thirds that observed per substation (Tables 24 and 25), suggesting that full species coverage is not achieved in a single still. The average number of species observed per substation is 42 and 47% for biotopes 3 and 4 respectively, but only 22% for biotope 6. These percentages are similar when looking at the number of families identified (Supplemental Table SB2).

Table 24. Average number of species, genera etc per still.

			Average number per still						
Biotope	**Code**	**#Sub Stations**	**Species**	**Genera**	**Families**	**Orders**	**Classes**	**Phyla**	**Kingdoms**
CR.HCR.FaT.CTub.Adig	1	4	18.5	19.3	20.0	15.6	11.1	8.0	2.1
CR.HCR.XFa.SpAnVt	2	1	33.5	35.5	33.5	20.0	12.0	8.0	2.0
CR.MCR.EcCr.CarSp.Bri	3	13	17.5	18.4	19.0	13.0	10.1	7.3	1.8
CR.MCR.EcCr.CarSp.PenPcom	4	11	25.3	27.4	26.6	16.5	11.7	8.2	1.5
IR.HIR.KFaR.LhypR.Pk	5	1	17.0	19.0	18.0	17.0	12.0	9.0	3.0
SS.SCS.OCS	6	14	7.6	7.9	8.2	6.4	5.6	4.7	1.3
SS.SMx.OMx	7	4	10.0	11.1	11.6	8.1	7.1	5.1	1.0
SS.SSa.OSa	8	1	3.5	3.5	3.5	3.0	2.5	2.0	1.0

Table 25. Average number of species per biotope, per substation and per still.

			per Biotope	per Station		per Still		
Biotope code[1]	**# Stations**	**# Stills**	**Avg # Species**	**Avg # Species**	**As % of Biotope**	**Avg # Species**	**As % of Biotope**	**As % of Station**
1	4	8	41	22	*53*	19	*45*	*85*
2	1	2	45	45	*100*	34	*74*	*74*
3	13	49	70	30	*42*	17	*25*	*59*
4	11	39	87	41	*47*	25	*29*	*62*
5	1	1	17	17	*100*	17	*100*	*100*
6	14	30	53	12	*22*	8	*14*	*65*
7	4	7	37	15	*40*	10	*27*	*68*
8	1	2	5	5	*100*	4	*70*	*70*
total	49	138	103	25	*24*	17	*17*	*70*

[1]See Table 20 for details.

Species accumulation curves

Figure 8 shows species accumulation curves for the five biotopes that have four or more stations. The curve for biotope 6 is flattening out only very slowly, suggesting that many stations are needed to cover all the species belonging to biotope 6. This is because the average number of species per substation is only 22% of that of the biotope (see previous paragraph), suggesting that species are sparse for this biotope.

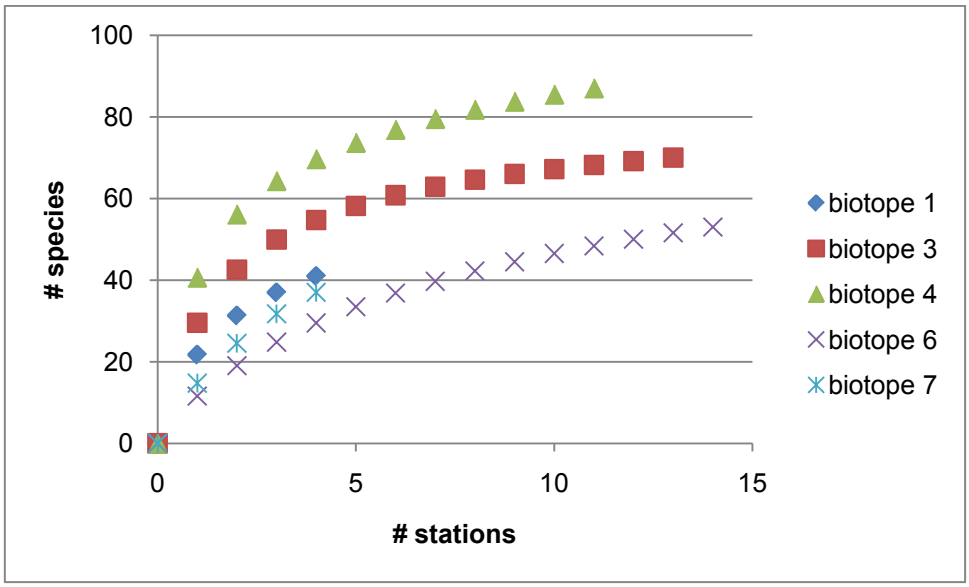


Figure 8. Average number of species observed when we have 1, 2, or more stations. Results are shown for biotopes that were present at four or more stations. See Table 20 for biotope codes.

Estimated total number of species

Based on the Chao index the total number of species for each biotope was estimated. As we have incidence data, this could only be done for biotopes with two or more stations. Results are presented in Table 26. Biotopes 3, 4 and 6 show the largest species richness with an estimated total number of species ranging from 71 to 101.

Table 26. Observed and estimated (based on Chao) number of species, genera and families for biotopes that have 4 or more stations.

	Biotope code[1]	1	3	4	6	7
	# Stations	4	13	11	14	4
Species	**Observed**	41	70	87	53	37
	Estimated	54	80	101	71	55
Genus	**Observed**	44	74	89	52	41
	Estimated	60	86	98	75	61
Family	**Observed**	43	64	75	51	40
	Estimated	59	72	78	72	57

[1]See Table 20 for biotope codes.

Number of stations needed per biotope to achieve a given coverage

More stations will allow for detection of more species albeit that we are dealing with the law of diminishing returns. Up to 9 stations per biotope are needed to sample 80% of the species (Table 27). The exception is biotope 6 which would require 21 stations. Results are similar when looking at genera or families.

Table 27. Number of stations needed per biotope to ensure that 70 to 95% of the total number of species, genera or families are sampled.

	Biotope code[1]	1	3	4	6	7
	% sampled					
Species	70	3	4	4	12	5
	75	4	6	6	16	7
	80	5	8	8	21	9
	85	8	11	11	30	12
	90	12	17	17	48	20
	95	25	36	36	100	41
Genus	70	4	5	3	15	5
	75	5	6	4	19	6
	80	6	8	5	25	9
	85	9	12	7	35	12
	90	14	19	11	56	19
	95	29	39	24	119	41
Family	70	4	4	2	13	4
	75	5	5	2	17	6
	80	6	6	3	23	7
	85	9	9	4	33	11
	90	14	14	6	52	17
	95	29	30	13	109	35

[1]Calculations were only performed for biotopes having 4 or more stations (note that these calculations are not possible for incidence data when we have one station only). See Table 20 for details biotope codes.

Figure 9 and Table 28 summarise the numbers of species observed for substations that had seven or more stills. From Figure 9 we see that the law of diminishing returns comes into play. When adding a third still or a fourth still we gain five and four species on average respectively, whereas adding a seventh still to six existing stills would only gain two species on average. These data also indicate that on average 53 species are estimated to be present per substation in biotope 3 and 51 species per substation in biotope 4. The estimated numbers of species for the entire biotopes are 80 and 101 respectively (Table 26), indicating that a single station (even if monitored in great detail) would not represent the full diversity of the biotope.

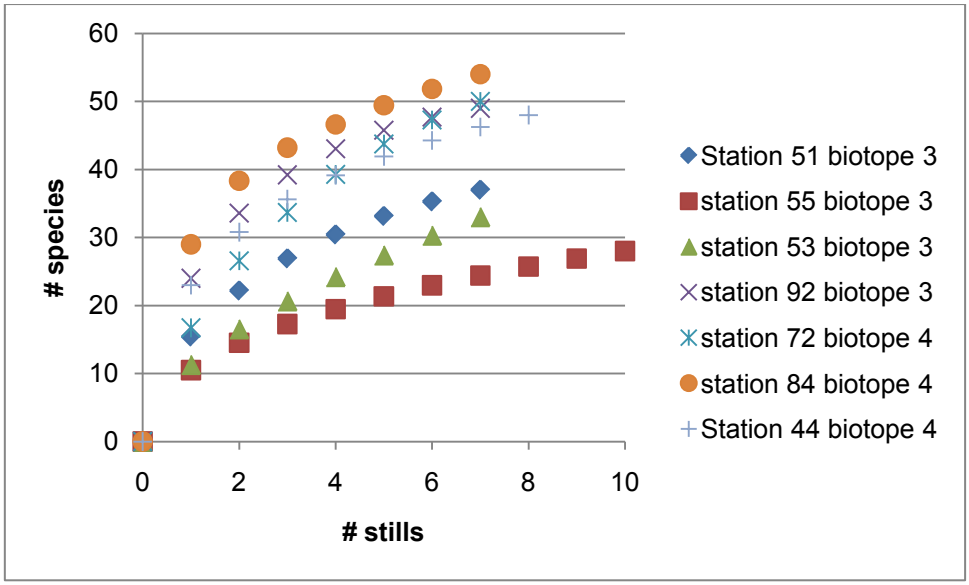


Figure 9. Average number of species observed when we have 1, 2, or more stills. Results are shown for stations that had 7 or more stills from the same biotope. The estimated total number of species was 51, 39, 60, 60, 78, 65 and 60 for stations 51, 55, 53, 92, 72, 84 and 44 respectively. See Table 20 for biotope codes.

Table 28. Number of species observed and estimated richness for four substations from biotope1 3 and three substations from biotope 4. Also shown the number of stills needed to ensure that 50-95% of the total species for the corresponding substation are sampled.

	Station 51 Biotope 3	Station 55 Biotope 3	Station 53 Biotope 3	Station 92 Biotope 3	Station 72 Biotope 4	Station 84 Biotope 4	Station 44 Biotope 4
# Stills	7	10	7	7	7	7	8
Estimated	51	39	60	60	78	65	60
Observed	37	28	33	49	50	54	48
% Covered							
50	2.7	4.1	5.9	1.6	3.9	1.5	2.1
60	4.0	6.1	8.8	2.4	5.9	2.3	3.2
70	6.2	9.5	13.7	3.7	9.2	3.6	4.9
75	8.0	12.3	17.6	4.7	11.8	4.6	6.3
80	10.7	16.3	23.4	6.3	15.7	6.1	8.4
85	15.2	23.2	33.2	9.0	22.2	8.7	11.9
90	24.1	36.8	52.7	14.2	35.3	13.8	18.9
95	50.8	77.6	111.2	30.1	74.6	29.2	39.9

[1]See Table 20.

To sample 80% of the species we would need 6 to 23 stills per substation (i.e. per biotope per tow) (Table 28), but given the preceding findings it seems more sensible to aim for sampling 50% of the species, which would require 2 to 4 stills per station per biotope.

Number of stations needed per biotope to detect changes in species richness

Table 29 shows how, if we want to detect a change in species richness per substation (i.e. per biotope type per station) of 10 or more, then 21 stations would be needed. This number of stations is similar for monitoring changes in the number of genera or families. Changes in

the number of orders found per station would require fewer stations (but as the number of orders is much lower we are less likely to see a decrease by 10 orders than by 10 species).

These calculations assume that the sampling effort per station is similar to that employed for the current data (i.e. if for the current data 3 stills were analysed per substation then the calculations above assume that future samples will also consist of 3 stills per substation).

Table 29. Number of stations needed per sediment or biotope to detect a change of 2, 4, 6,… 20 species (genera, families etc) in the average species (genera, families etc) richness per station. The between station spread (i.e. standard deviation) in the number of species, genera etc is given in the second column. The numbers in the body of the table are the number of stations needed per sediment or biotope. Based on power of 80% at the 5% significance level.

		Change in number of species, genera etc						
	Spread	**2**	**4**	**6**	**8**	**10**	**15**	**20**
Species	11.3	500	126	57	33	21	10	7
Genus	12.5	614	155	69	40	26	12	8
Family	11.2	496	125	56	32	21	10	7
Order	6.2	151	39	18	11	8	4	3
Class	3.3	45	12	6	4	4	3	2
Phylum	1.8	14	5	3	3	3	2	2

Diversity indices

The relationship between species richness, phylogenetic diversity (PD) and taxonomic diversity (calculated as either the sum of the path lengths connecting pairs of species (TD01sum) or as the average path length connecting pairs of species (TD01avg)) is shown in Figure 10. As before, a strong association is seen between species richness, PD and TD01sum.

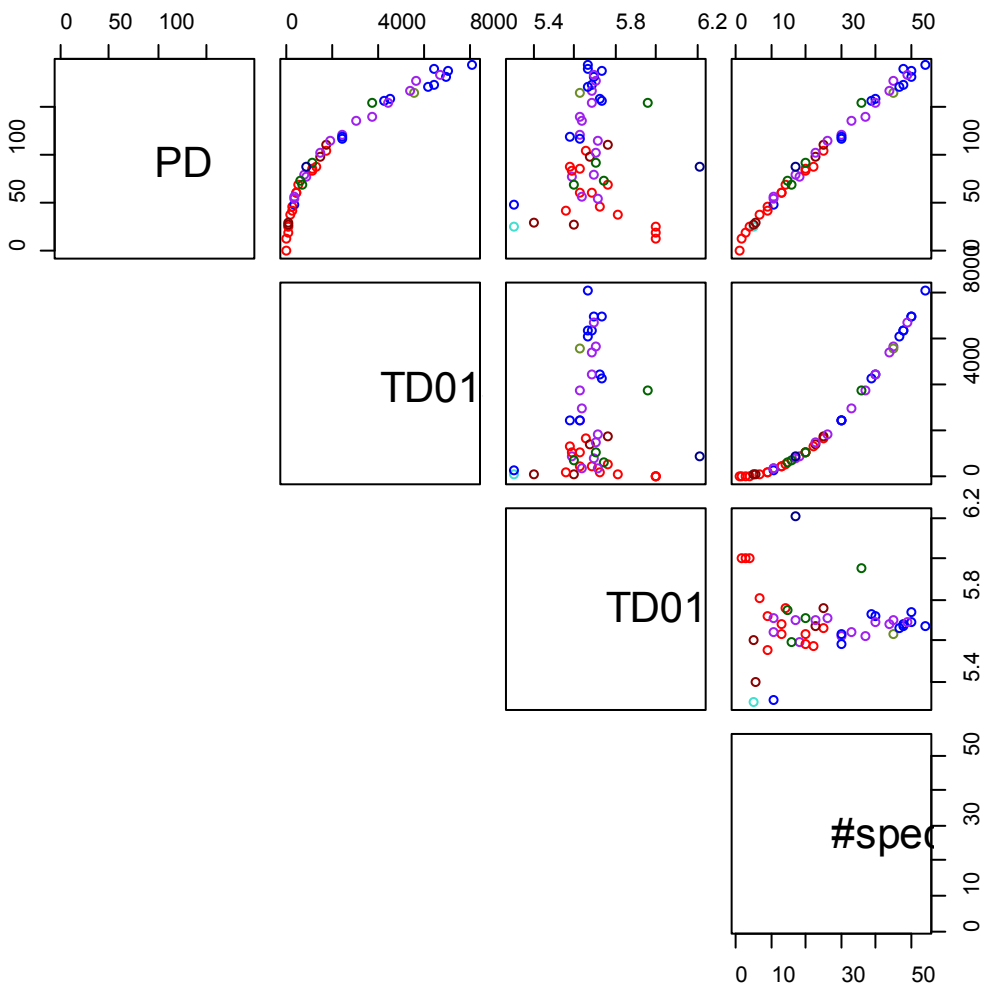


Figure 10. Comparing phylogenetic diversity (PD) and taxonomic diversity indices. (TD01sum: sum of distances between pairs of species; TD01avg: average distance between pairs of species), based on species incidence data observed per substation (i.e. per station per biotope). Also shown is the species richness. Colours reflect the different biotopes (see Supplemental Table SB1).

Table 30 summarises the phylogenetic diversity for each biotope. Biotopes 3 and 4 show the highest γ diversity, followed by biotope 6. These biotopes contain the largest number of stations and this may have contributed to the high diversity. Therefore the phylogenetic diversity was also calculated for each substation separately (α diversity), which was then averaged per biotope. Now biotope 6 is among the least diverse biotopes, whereas biotope 2 shows the largest diversity per station. The same pattern is observed when looking at the phylogenetic diversity per still. The average phylogenetic diversity per still is less than that per substation, suggesting that one still does not cover the full species richness of the substation. Beta diversity (i.e. the difference between biotope diversity and station diversity expressed as % of biotope diversity) was 46, 50 and 73% for biotopes 4, 3 and 6, respectively. Similar patterns were observed when looking at observations identified to at least the family level (Supplemental Table SB3). Although not reported in detail here, similar findings were observed for taxonomic diversity TD01sum.

Table 30. Phylogenetic diversity (PD) for each biotope based on data identified down to the species level. PD was also calculated for each station and then averaged per biotope. Likewise, PD was calculated for each still and then averaged per biotope.

Biotope code[1]	# Stations	# Stills	Biotope PD γ div.	per Station Avg PD α div.	per Station As % of Biotope PD 100 - β div.	per Still Avg PD	per Still As % of Biotope PD	per Still As % of Station PD
1	4	8	166	97	*58*	85	*51*	*88*
2	1	2	163	163	*100*	124	*76*	*76*
3	13	49	240	119	*50*	77	*32*	*64*
4	11	39	282	153	*54*	102	*36*	*67*
5	1	1	87	87	*100*	87	*100*	*100*
6	14	30	191	52	*27*	36	*19*	*69*
7	4	7	150	66	*44*	44	*29*	*67*
8	1	2	24	24	*100*	18	*75*	*75*
combined	49	138	337	100	*30*	74	*22*	*74*

[1]See Table 20 for details.

When taxonomic diversity is expressed as the average path length between any two species or any two families (shown in Table 31 and Supplemental Table SB4, respectively) there are no major differences between the biotopes. Nor are there any differences between taxonomic diversity based on the species composition per biotope, station, or still. This would suggest that although a single still only contains a subset of the species associated with the corresponding biotope, this subset of species is not confined to one family or one class only.

Table 31. Taxonomic diversity expressed as average distance between pairs of species (TD) for each biotope based on data identified down to the species level. PD was also calculated for each station and then averaged per biotope. Likewise, PD was calculated for each still and then averaged per biotope.

Biotope	Code	# Stations	# Stills	TD Biotope	Avg TD per Station	Avg TD per Still
CR.HCR.FaT.CTub.Adig	1	4	8	5.92	5.75	5.71
CR.HCR.XFa.SpAnVt	2	1	2	5.63	5.63	5.58
CR.MCR.EcCr.CarSp.Bri	3	13	49	5.69	5.67	5.66
CR.MCR.EcCr.CarSp.PenPcom	4	11	39	5.67	5.64	5.63
IR.HIR.KFaR.LhypR.Pk	5	1	1	6.21	6.21	6.21
SS.SCS.OCS	6	14	30	5.70	5.74	5.54
SS.SMx.OMx	7	4	7	5.74	5.61	5.57
SS.SSa.OSa	8	1	2	5.30	5.30	5.65
Combined		49	138	5.78	5.69	5.63

Phylogenetic diversity and species richness show a positive association with the percentage of bedrock, boulders, and cobbles, and a negative association with the percentage of sand (see Figure 11), with up to 25% of the observed variation in species richness or phylogenetic diversity explained by sediment type. Taxonomic diversity, expressed that average path length between pairs of species, did not show any significant association with sediment composition.

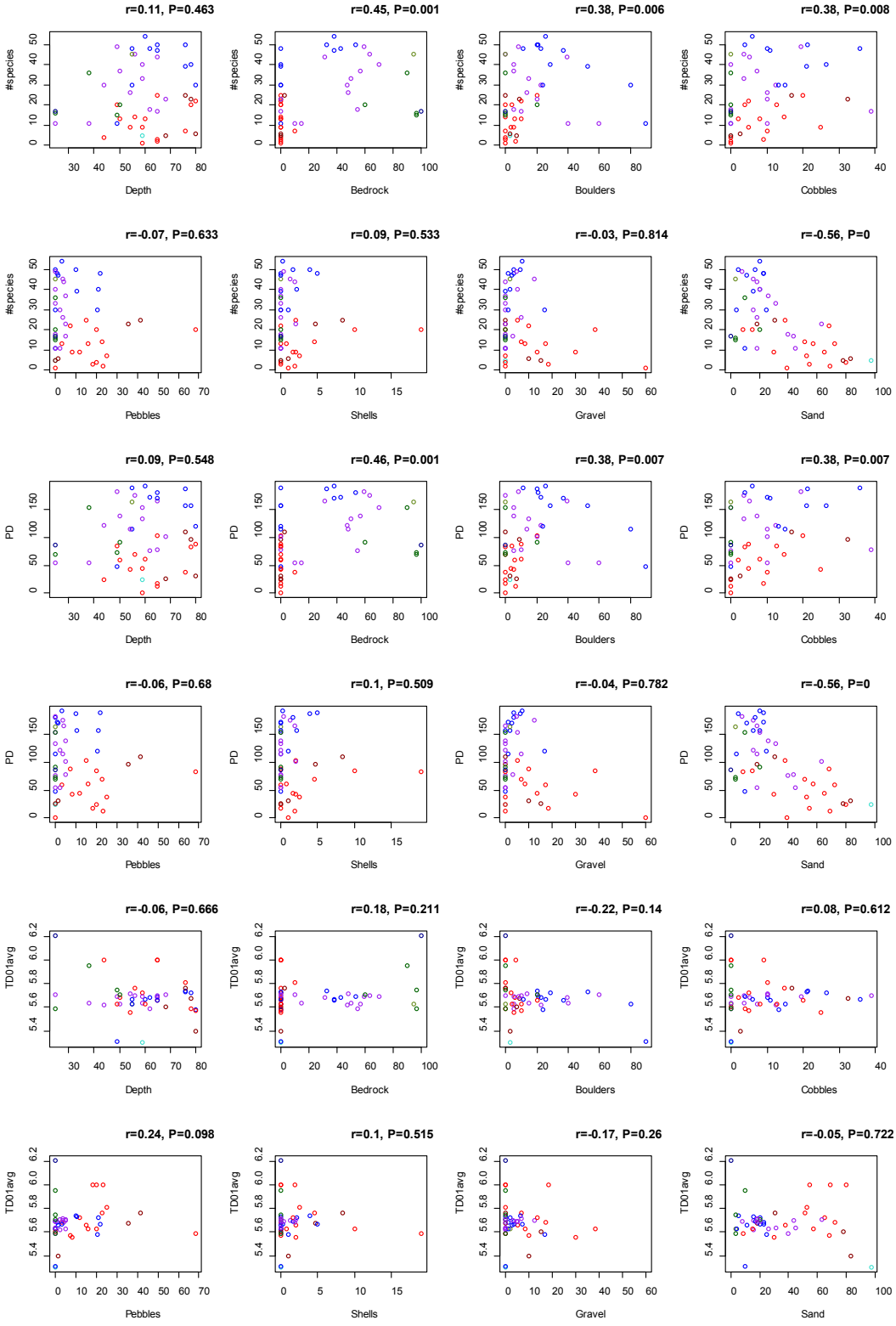


Figure 11. Relationship between sediment characteristics and species richness (first two rows), phylogenetic diversity (middle two rows) and taxonomic diversity (defined as the average path length between any pair of species) (last two rows). Based on species data. Colours reflect the different biotopes (see Supplemental Table SB1). Spearman rank correlation (r) and its P-value (P) are shown in figure headers.

4.3 Principal coordinates analysis

Supplemental Figure SB2 shows the β diversity based on ordination plots from principal coordinates analysis. It shows the similarities between substations based on observations specified to species level, genus level, etc. On the whole, the substations tend to separate according to biotope type. The pattern of separation is similar irrespective of which level of the phylogenetic hierarchy (i.e. species, genus, family) we are looking at (please note that the configuration may be arbitrarily mirrored from left to right or from top to bottom).

Figure 12 shows the principal coordinates analysis for the individual stills. The separation according to biotope is similar to that observed in Supplemental Figure SB2 (keeping in mind arbitrary mirroring of configuration). Generally, stills obtained from the same station and biotope tend to show similar species composition (see for example station 55 shown in purple or station 51 shown in red). This is not always the case however; station 53 purple shows different species compositions for each of the stills, indicative of a diverse and patchy habitat.

Figure 13 (species) and Supplemental Figure SB3 (family) show the relationship between the station ordinations and their corresponding sediment characteristics. Stations with a large percentage of bedrock tend to cluster together. To some extent this is also seen for cobbles and pebbles. Supplemental Figures SB4 and SB5 show similar patterns for the stills data.

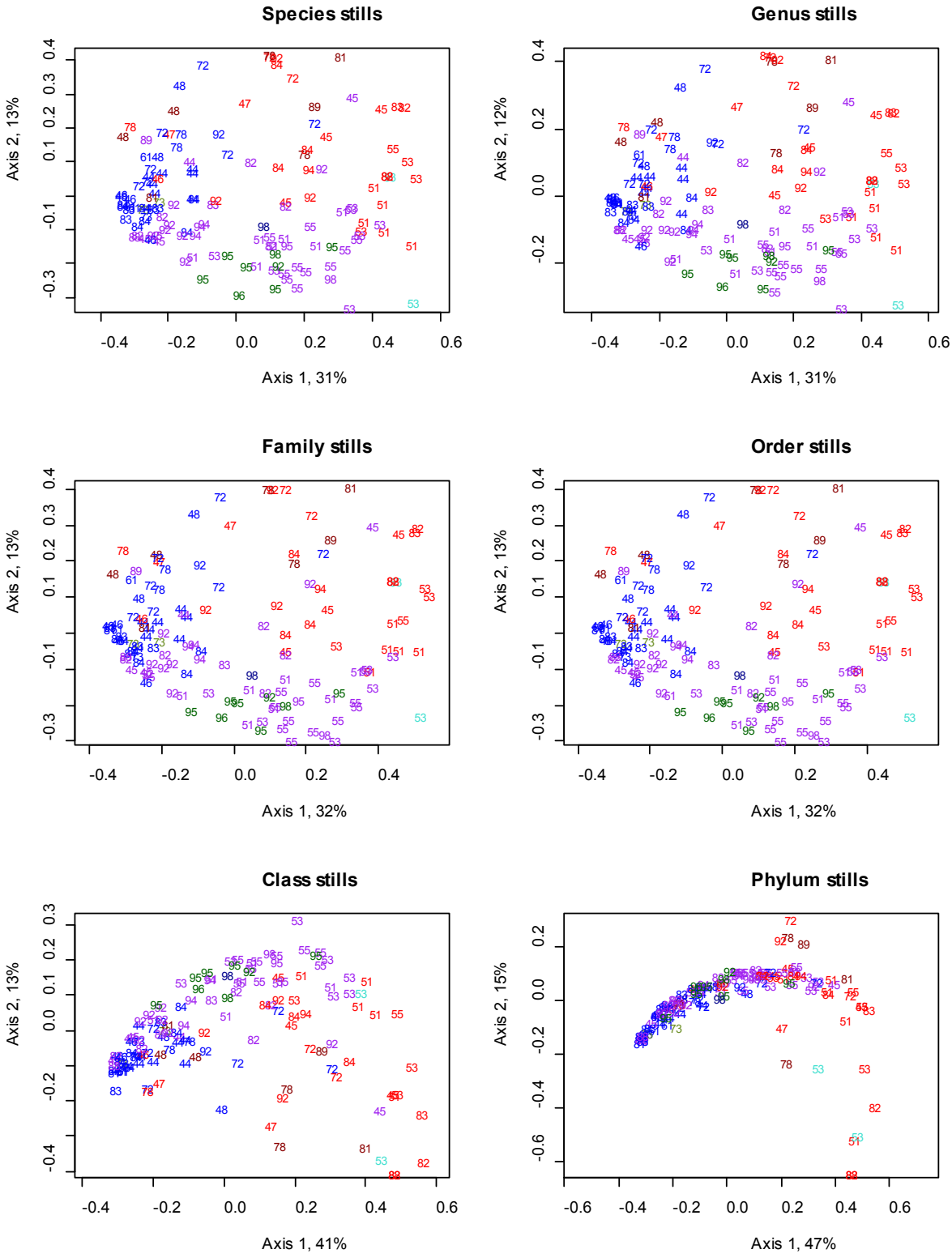


Figure 12. PCO analysis for incidence data observed at each substation. Each substation is be presented by its corresponding station number and colours reflect the different biotopes (see Supplemental Table SB1). As several stations cover more than one biotope, station codes can appear more than once. Each graph is based on all observations that were identified to at least the corresponding phylogenetic level in its title.

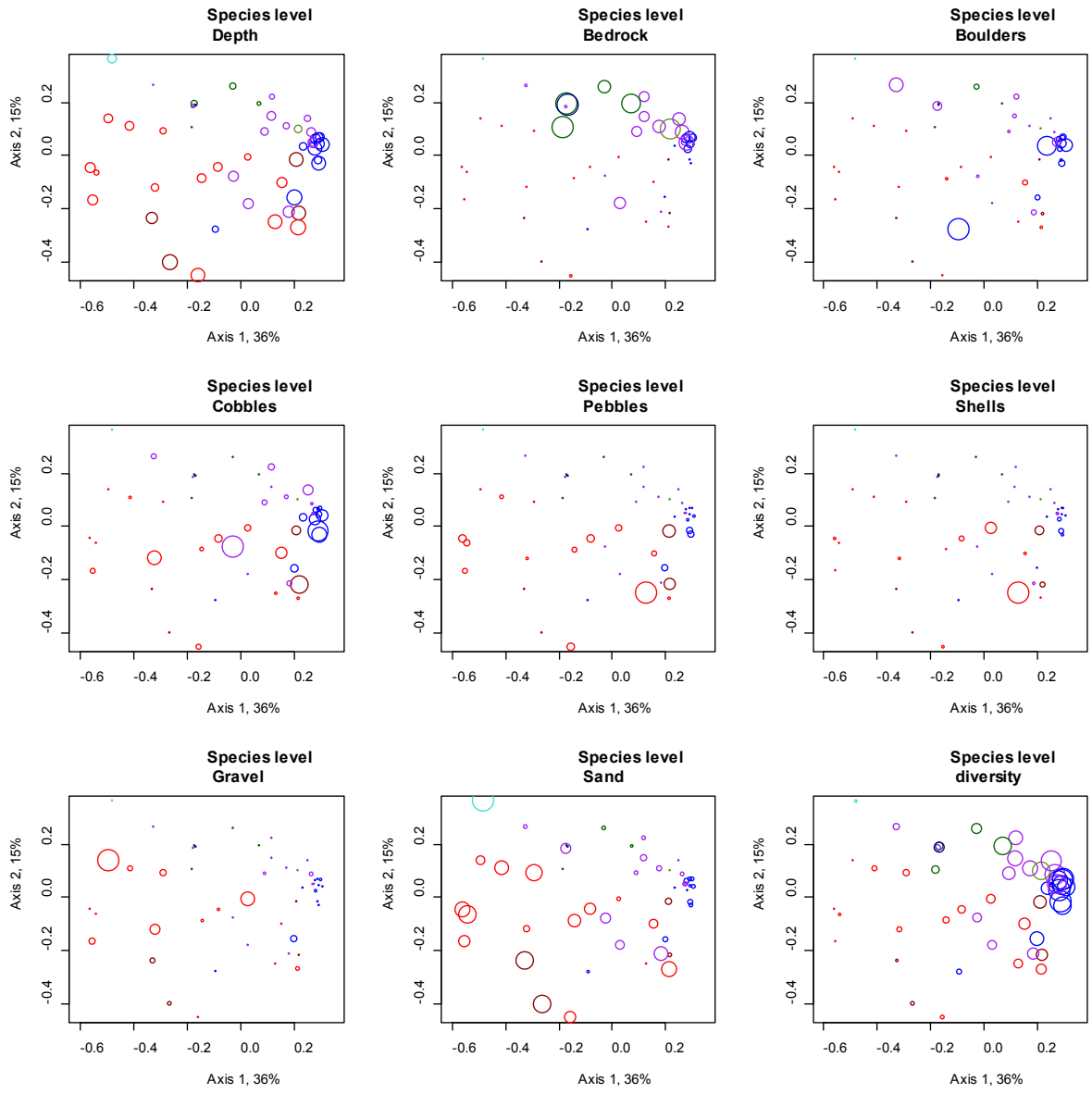


Figure 13. The ordination species data per substation is shown here, with each substation represented by a circle. The diameter is an indication of depth, percentage bedrock etc. The final plot shows phylogenetic diversity. Colours reflect biotopes (see Supplemental Table SB1). Based on data identified to species level.

Are substations that are geographically close more similar than stations further apart?

Figure 14 shows the substation ordination based on species data in enlarged format. Station 53, which covers three biotopes, shows different species compositions for the three biotopes. Likewise, station 55 shows different species composition between the red and purple biotopes. On the other hand, station 98 shows similar species composition between the three biotopes.

Examples of stations that are far apart geographically but share similar species composition are stations 55 and 82 (red biotope) and stations 92 and 45 (purple biotope).

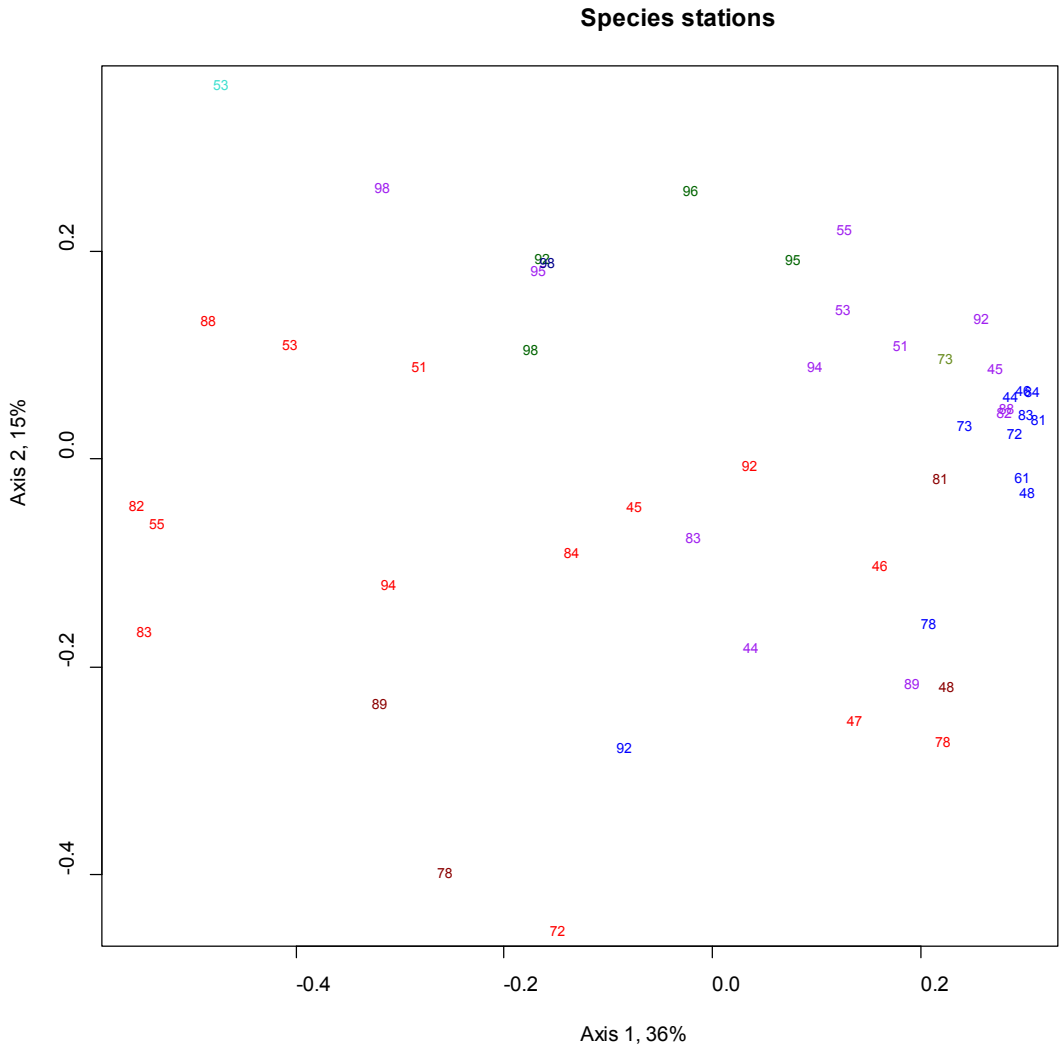


Figure 14. Principal coordinates ordination for data identified to the species level per substation is shown in an enlarged form (this is the same figure as the first sub-plot in Supplemental Figure SB2). Numbers refer to stations and colours reflect biotopes (see Supplemental Table SB1).

Are stills from the same substation more similar than stills from different substations?

Figure 15 shows the stills ordination based on species data in enlarged format. The stills are coloured according to biotope and we can see that there is no strict separation between the biotopes. Stills from the same substation (and hence the same biotope) have a tendency to be more similar than stills from different substations. For example, station 94 purple biotope shows similar species composition for the three stills, and this composition is quite different from that observed from station 94 red biotope. On the other hand, stills from station 72 blue biotope show a large spread. There are also examples of stills from the same station but from different biotopes to be similar in species composition. For example, one of the red biotope stills of station 53 is close to the purple biotope stills from the same station. Likewise, one of the purple biotope stills of station 44 is close to the blue biotope stills from the same station.

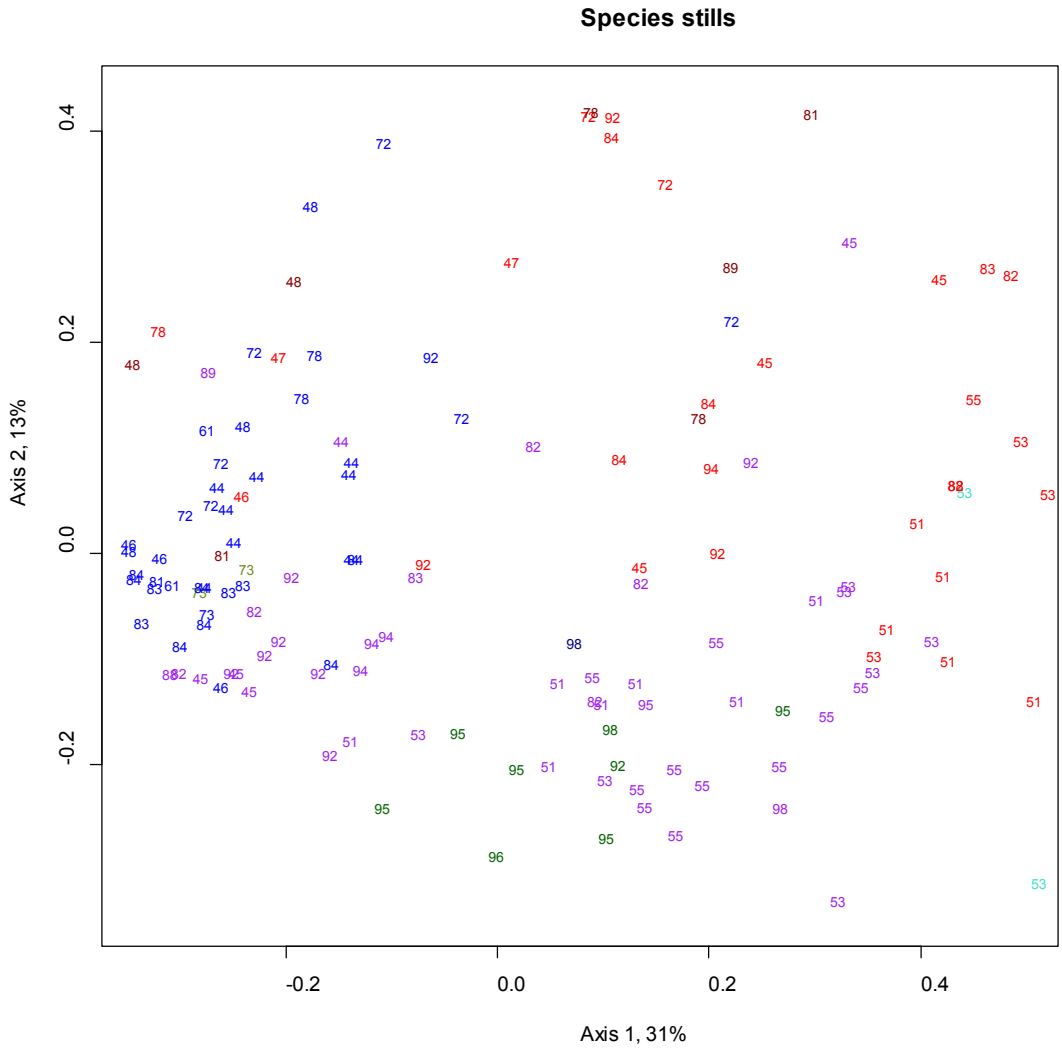


Figure 15. Principal coordinates ordination for data identified to the species level per still is shown in an enlarged form (this is the same figure as the first sub-plot in 12). Numbers refer to stations and colours reflect biotopes (see Supplemental Table SB1).

4.4 Conclusions

- Despite only three-quarters of the data having been identified to the species level, similar results are obtained irrespective of whether we are looking at species, genus, or family level of the phylogenetic tree.

- One still does not cover the full diversity (either based on phylogenetic diversity or species richness) of its corresponding station, and on average a still covers no more than about two-thirds of the diversity observed at the station.

- The diversity (either based on phylogenetic diversity or species richness) observed per station is only about half that of the corresponding biotope. For example, 87 species were observed for biotope 4 (γ diversity), but only an average of 41 species were observed per station (α diversity) within this biotope. For biotope 6 this was even less with one station only covering, on average, 27% of the diversity.

- Although there was a statistically significant correlation between phylogenetic diversity or species richness with sediment type, this relationship was not strong with only up to 25% of the variation in species richness or diversity explained by sediment type.
- To sample at least 80% of the species present at a given station at a given biotope, we would need 6 to 23 stills per station.

- The number of stations required to sample 80% of the species would require five to nine stations for biotopes 1, 3, 4 and 7. For biotope 6, 21 stations would be needed. This assumes having two to four stills (i.e., the current data) per station per biotope.

- In practice, it may not be possible to analyse up to 23 stills per station. If a choice has to be made between more stations or more stills per station, then having more stations is preferred as this is more likely to enhance coverage of the species associated with the biotope.

- Note: Although a coverage of 80% has been used to illustrate the corresponding number of stations required, this is an arbitrary choice and it is up to JNCC to decide what is desirable.

5 Comments and recommendations

5.1 More stations or more intensive sampling per station?

The aim of the monitoring scheme is to establish the condition, in terms of species diversity, of biotopes (Solan Bank) or sediments (Rock Unique) of interest. In terms of sampling effort, would it be preferred to monitor only a handful of stations but sample each of these stations extensively in order to get a full picture of the species diversity for each of these stations, or would we be better to monitor many stations but spend less effort per station? This question can be addressed by looking at the estimated species richness per station and compared it against the estimated species richness of the corresponding biotope or sediment.

Species richness of a grab sample versus station versus sediment (Rock Unique)

The data for Rock Unique were obtained from grab samples (one grab sample per station), and the abundance data indicate that the number of species observed in a grab sample amounts to approximately 50% of the estimated total number of species at the corresponding station. The estimated number of species per station is approximately 20 to 37% of the estimated total number of species for the sediment. Clearly, data from a single station, even if all species were recorded, would not cover the diversity of the corresponding sediment.

Recommendation: if a choice had to be made between collecting more grab samples per station and increasing the number of stations, then increasing the number of stations would be preferred.

Species richness of a set of stills versus station versus biotope (Solan Bank)

Currently, 2-4 stills taken at 1 min intervals are obtained per substation (i.e. per station per biotope). The cost of obtaining additional stills from a given station is negligible, analysing these stills for species diversity is time-consuming and expensive however. How to balance this against the need for observing the full species composition? How many stills do we need per station per biotope? The Solan Bank data consist of incidences and Chao's index for incidence data was used to estimate the total number of species per substation. Because it is based on the number of stills within a station for which a species is present, this calculation can only be performed when we have at least 7 or so stills per substation. This was the case for 7 out of the 49 substations, four of which came from biotope 3 with the remaining three substations from biotope 4.

The data from these seven substations indicate that 2 to 4 stills would include 50% or more of the species present at the station. For biotope 3 the estimated total number of species per station is 53 on average, whereas for the biotope as a whole the estimated total number of species is 80, i.e. a single station, even if all species at this station were sampled, would cover no more than 60% of the total number of species for this biotope. For biotope 4 the estimated total number of species per station (51 on average) would be only half that of the biotope (estimated total number of species for the biotope as a whole was 101). Clearly these results indicate that it is important to have several stations per biotope. If a choice has to be made between more stations or more stills per station, then having more stations is preferred as it is more likely to enhance coverage of the complete set of species associated with the biotope.

Recommendation: If a choice has to be made between monitoring more stations or more stills per station, monitoring more stations is preferred.

How many stills per station would be needed? The law of diminishing returns applies to the total number of species observed when we have more stills. Based on the limited data where

we have 7 or more stills per station per biotope, indications are that 2 to 4 stills would cover 50% or more of the species present at the station. On average, one still would identify 19 species. A second still would add 8 species, whereas a third and fourth still would add another 5 and 4 species respectively. The gain per additional still (in terms of uncovering more species) is diminished when analysing more stills and adding a 7th still would uncover only 2 additional species to those observed in stills 1 to 6.

Recommendation: Where possible, a minimum of 2 stills per station per biotope should be analysed. There is relatively little gain to be had from analysing more than 6 stills per station per biotope.

Number of stations needed

As pointed out above, both Solan Bank and Rock Unique show that species richness observed at a single station does not cover the species richness of the corresponding sediment or biotope (even if all species at a given station were monitored). In other words, β diversity is substantial. The Results Section and corresponding Tables and Figures illustrate how many stations would be required to ensure that 80% coverage of all the species associated with the biotope or sediment type of interest. The CR biotopes of Solan Bank would require no more than 5 to 8 stations per biotope to sample 80% of the total number of species at each of these biotopes. For the coarse, mixed and sandy sediments of Rock Unique and the offshore coarse sediment (SS.SCS.OCS) of Solan Bank, the number of stations is much higher, with 21 - 37 stations per sediment required to sample 80% of the total number of species associated with these sediments. This is because these sediments are made up of multiple biotopes.

The choice of 80% is arbitrary and it is up to JNCC to decide what would be appropriate. The Tables provided can be used to derive the number of stations needed for alternative coverage percentages.

It should be kept in mind that the estimated number of stations needed to achieve a given coverage should be interpreted loosely, as this is largely based on extrapolation of the species accumulation curves beyond the actual data.

Are biotopes (Solan Bank) less diverse than sediment types (Rock Unique)?

For those biotopes and sediments that had 10 or more stations each (namely Solan Bank biotopes 3, 4 and 6 and Rock Unique sand, coarse and mixed sediments) we find that the number of observed species per Solan Bank biotope ranged from 53 to 70, whereas for the Rock Unique sediments it ranged from 152 to 209. This is probably a reflection of sediments being composed of more than one biotope and hence increasing species diversity. These findings are also reflected in the numbers of stations needed to sample 80% of the total number of species being less for the Solan Bank biotopes (as discussed in the preceding paragraph).

When looking at the average number of species per station we find that this ranged from 12 to 41 for the Solan Bank biotopes and from 21 to 37 for the Rock Unique sediments, i.e. it appears that despite the use of different sampling methods (stills, grab samples) and differences in habitats the number of species per station is not all that different for the sand, coarse and mixed sediments for Rock Unique and Solan Bank biotopes 3, 4 and 6.

5.2 Diversity indices and species richness

First, a brief explanation of the various diversity indices is given, followed by further comments.

Summary of indices

In what follows, assume that we have a biotope with several stations, with one set of observations per station (such as results from one grab sample or one video still). The observed species richness is simply a count of the total number of species, and can be obtained for any individual data set, irrespective of whether these are incidence, abundance or biomass data. The total (observed and unobserved) number of species is estimated based on Chao's index. Full details are given in the Methodology section but essentially this approach tries to estimate the number of missed species based on the number of species in our data set for which we only have 1 or 2 observations. In the case of incidence data we need several data sets (stations), whereas for abundance data this can be done for each data set (station) individually. The Shannon and Simpson indices are widely used measures of species diversity that combine species richness with a measure of evenness, i.e. how evenly the numbers of observations are spread among species. Data showing a more even spread are regarded as more diverse.

Indices can be calculated for each station separately (α diversity) or from combining the data from all stations to obtain the diversity for the biotope (γ diversity)

Neither species richness nor the Shannon and Simpson indices take the complexity of the phylogenetic tree into account. The phylogenetic diversity index tries to address this by looking at the number of branches in this tree. The taxonomic diversity index tries to address this by looking at distances between pairs of species, i.e. the number of branches that separate a species pair. Two versions of the taxonomic diversity were investigated in current report; one version looks at the sum of distances between all species pairs (TDsum), and the second version looks at the average distance between species pairs (TDavg). Diversity indices can be calculated for each station individually (α diversity) or data from all stations can be combined to calculate the diversity of the biotope (γ diversity). Table 32 summarises which quantity can be obtained from which type of data.

Table 32. Assuming several stations within a biotope, with one data set per station, this table summarises which quantities can be obtained from which type of data.

	Type of data		
	Incidence (0/1)	**Abundance (counts)**	**Biomass**
Observed species richness per biotope	Yes	Yes[1]	Yes[1]
Observed species richness per station	Yes	Yes[1]	Yes[1]
Estimated species richness per biotope[2]	Yes	Yes	Yes[1]
Estimated species richness per station[2]	No	Yes	No
Shannon index of diversity[3]	No	Yes	Yes
Simpson's index of diversity[3]	No	Yes	Yes
Phylogenetic diversity[3]	Yes	Yes[1]	Yes[1]
Taxonomic diversity[3,4]	Yes	Yes	Yes

[1]Treated as incidence data
[2]Chao's index. This index has slightly different versions for incidence and abundance data (details given in Methodology Section).
[3]Can be calculated for one station (α diversity) or for the biotope (γ diversity), calculation is the same.
[4]This index is based on distances between pairs of species (incidence data) or pairs of individuals (abundance data, biomass data).

Comments on diversity indices

Some simple examples comparing the Shannon and Simpson indices are given in the Methodology Section, and these show that the Simpson index puts more emphasis on observations being distributed evenly over species, even if it means fewer species. Nevertheless, for a given number of species the two indices show similar behaviour.

For both the Solan Bank and Rock Unique data sets the phylogenetic and taxonomic (TDsum) diversity indices per station show a strong correlation with the number of species observed. Although at first sight there may seem to be little benefit in calculating these indices in addition to species richness, it does contain some useful additional information. For example, from Figure 9 we can see that increasing the number of species from 10 to 40 results in a change in phylogenetic diversity from 50 to 150. If all of these 30 additional species had belonged to the genera to which the first 10 species belong then the phylogenetic diversity would have increased by only 30 units. In reality it increased by approximately 100 units which indicates that additional species cover new (that is, not covered by the first 10 species) genera, families and so on. The taxonomic diversity index (TDsum) shows a similar message.

Taxonomic diversity expressed as average path length between pairs of species (TDavg) shows quite a different behaviour. Unlike Shannon, Simpson, phylogenetic diversity and TDsum, this index does not necessarily increase when more species are being observed when our sampling effort is increased. This is because it is expressed as an average, so any effect of increased sample size or increased numbers of species has been taken out of this index. As such, the type of information obtained from this index is quite different, and is probably most useful when comparing diversity for a single still versus diversity at a station versus diversity of a sediment type or biotope. To illustrate, for the Solan Bank data (Table 31) the average distance between species pairs is similar irrespective of whether we look at all the species observed across the entire biotope, or species observed at a given station, or species observed in a given still. This implies that, although the number of species observed in a still is substantially less than that observed across the entire biotope, the species observed in a still are not confined to one branch of the phylogenetic tree only. In other words the species observed in a still appear to be a random selection of the species, families, orders etc observed across the biotope. Similar behaviour was observed for the Rock Unique data.

What if a rare species is of interest?

Diversity indices attempt to summarise the abundances of a large number of species into a single number. Now assume that we are interested in monitoring the abundance of a rare species that is threatened with extinction. Would a change in the abundance of this species be reflected in diversity indices? The answer is probably not. The reason for this is that diversity indices are based a summation of abundances over species, and a minor change in one species (a low abundance becoming even less) would be swamped by the contributions of the large number of remaining species to the diversity index.

Recommendation: if the status of a single species is of interest (perhaps because it is threatened with extinction, because it acts as an indicator species) then it is recommended that the abundance of the species is reported separately. Diversity indices should not be relied on to monitor changes in one species only.

5.3 Patterns in data

Ordination techniques revealed that species composition observed at the stations tended to group according to biotope (or type of sediment). This grouping was not strong, however, and overlap between biotopes (or sediments) was seen. Furthermore, species composition observed per still (Solan Bank) tended to be more similar when stills came from the same station and biotope, but again overlap with stills from other stations was seen. Furthermore, observations from stations in close proximity sharing the same biotope (sediment-type) tended to be similar in species composition.

Relating the ordination plots to sediment composition showed an association with % bedrock for the Solan Bank data. For the Rock Unique data, an association with the % sand and gravel was observed. In addition to relating ordinations to sediment composition, we also looked at correlations between diversity indices and sediment composition. For the Solan Bank data this showed a positive association between diversity and % bedrock, boulders, and cobbles, and the negative association with %sand. For the Rock Unique data a positive association was seen between diversity and % gravel, and the negative association with %sand. Although these associations were statistically significant, the strength of these relationships was relatively weak with no more than 10 to 25% of the variation in diversity explained by the sediment characteristics.

It should be noted that ordination plots are simplifications of the data. If we had only two species per station we could simply plot species A along the x axis and species B along the y axis, and distances between stations in this plot would reflect the true dissimilarity between the two stations. Ordination techniques are generalisations of this approach, and attempt to plot the stations such that stations that have similar species composition are plotted close together. Ordination techniques allow the high dimensionality (number of species) in the data to be reduced to a 2-dimensional plot, but as a consequence a certain degree of information is lost. The percentages given along the axes of the plots give an indication of how well the ordination represents the true dissimilarities. For the biomass data the species, genus and family ordinations were poor, representing only approximately 25% of the true underlying dissimilarities. Therefore these ordinations should be treated with caution. For the incidence and abundance data from Rock Unique the representation is slightly better, covering about a third of the true dissimilarities. For the Solan Bank data this is approximately 50%.

Some arching was observed in several of the ordination plots, which is probably due to an underlying ecological gradient; for example, stations from one end of the arch might show a large percentage of gravel, whereas stations at the other end of the arch might show a low percentage of gravel. This arching is generally a mathematical artefact and, as a consequence, distances between stations should be read ‘along the arch’ (i.e. imagine a straightening out of the arch). This does not change our overall findings.

The remit of the current report was limited to using exploratory techniques to identify any patterns or trends, and as such no formal statistical testing was performed to identify whether ordination plots showed statistically significant clustering according to sediments. Such in-depth statistical analyses were not within the scope of this contract.

5.4 Sampling schemes

This Section describes sampling strategies with their advantages and disadvantages.

The aim of any sampling scheme is to build up a picture of an area of interest. The samples should be obtained such that the information obtained from the samples is representative of that of the area. Furthermore, to be cost-effective, these samples should be as independent from each other as is possible. When monitoring biodiversity in a large area we face the problem of how to choose locations (stations), and how many stations. The three most

commonly employed sampling strategies are: random sampling, systematic sampling, and stratified sampling. In what follows, the pros and cons of each of these approaches are discussed.

Random sampling: for simplicity assume that the area of interest is a rectangle (random sampling is easily extended to other shapes), with the boundaries defined by the corresponding longitude and latitude coordinates. Using a random number generator we randomly generate a longitudinal coordinate and randomly generate a latitudinal coordinate, which then correspond to a sampling station. Each sampling station is obtained this way. See Figure 16A for an example.

Advantages: this method avoids bias, i.e. characteristics observed in the sample will be representative of characteristics of the entire site.

Disadvantage: it can lead to poor representation of the total area, especially if the total area is large (although this can be remedied by increasing the number of samples).

Systematic sampling: this consists of sampling along a grid at equidistant intervals. The first sampling location is determined at random (i.e. randomly generate a longitudinal coordinate and a latitudinal coordinate), but all other stations are then determined according to the grid. See Figure 16B.

Advantages: straightforward to implement, good coverage of total area.

Disadvantages: this approach can lead to bias, i.e. some patterns may be over- or underrepresented in the sample, especially if the grid misses an important biotope. This is illustrated in Figure 16D.

Stratified sampling: the site is made up of known habitats and the sampling effort is divided accordingly. The sampling effort can be based on proportional to the area of each habitat (Figure 16C), or it may be set to a minimum or maximum number of samples per habitat.

Advantages: all habitat of interest are covered.

Disadvantages: need to know in advance the habitat locations.

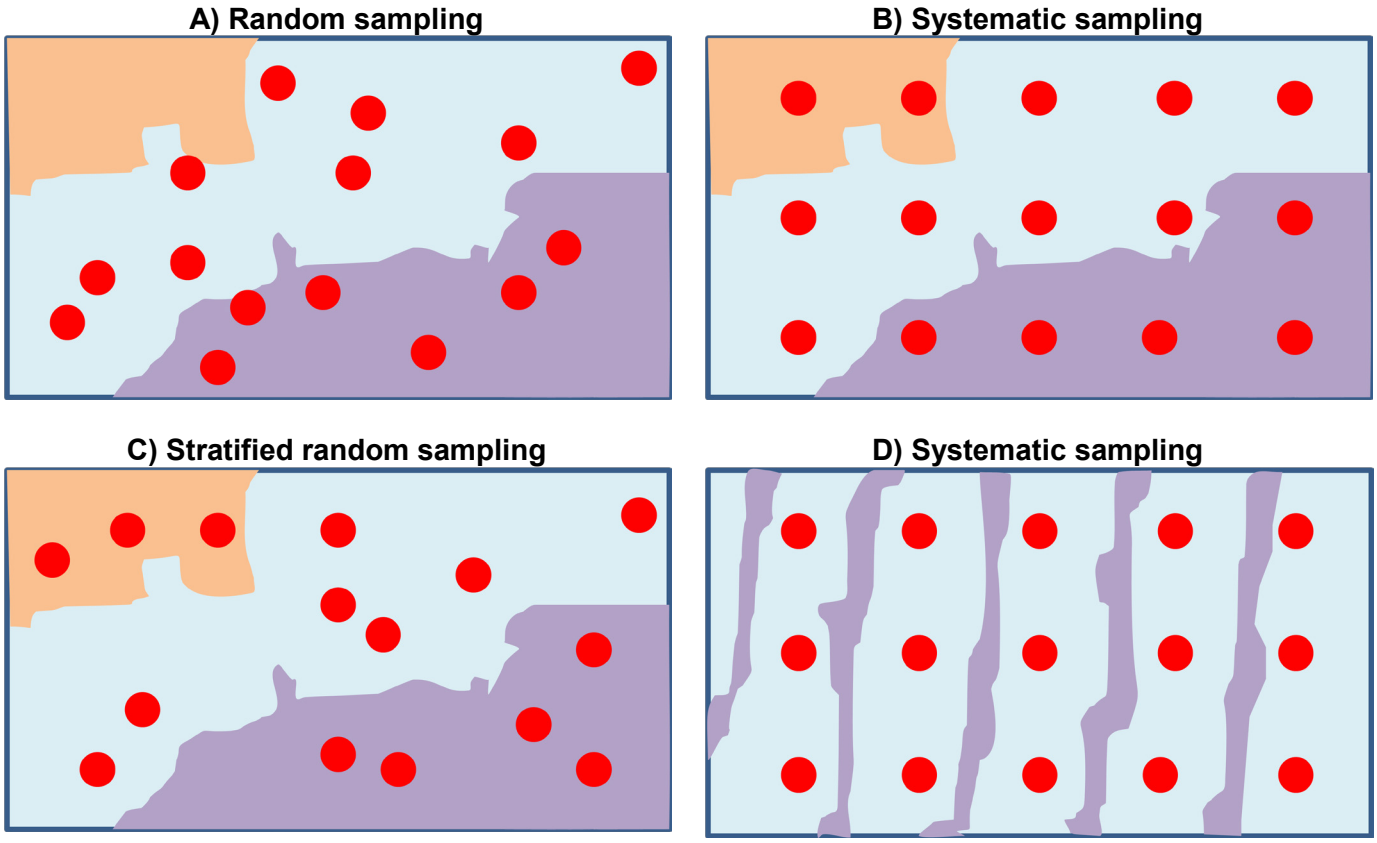


Figure 16. Examples of sampling schemes. Red dots denote sampling locations, the blue, purple and orange backgrounds denote different habitat types.

Transect sampling

Transect sampling can be used to ensure a good coverage of environments where conditions can change rapidly, or which are difficult to sample otherwise. Ideally the distance between stills should be such that observations on each still are as independent of each other as possible (to avoid pseudo-replication), but at the same time should ensure a good coverage of the environmental conditions. If the variogram analysis indicates that the spatial autocorrelation is large with respect to the length of the transect then we can either (i) design the sampling scheme to account for this, which may mean taking more transects with fewer stills on each; or (ii) use the existing sampling scheme but use statistical methods to account for the spatial autocorrelation at the analysis stage.

Recommendation: if the distribution of habitats within a site is known, then stratified sampling is recommended. This means that a predetermined amount of sampling effort is dedicated to each habitat. Sampling within the habitat can be based on random sampling or systematic (i.e. grid) sampling, where the latter is more likely to cover all the characteristics within the habitat.

5.5 Further comments

Species versus genera versus families

Only two-thirds to three-quarters of the data were identified down to the species level. This went up to 90% identified at the family level. Despite this considerable lack of data at the species level, findings (such as ordination patterns and diversity patterns between sediments or biotopes), were consistent across these three levels of the phylogenetic hierarchy. This is a consequence of the percentage of non-identified species per family being broadly similar

across families. If, on the other hand, the percentage of non-identified species is not evenly spread across families, we might expect diversity patterns at the family level to differ from those at the species level. What did differ was the number of stations needed to achieve a given percentage of species included in the sample, as 80% coverage (say) at the family level would require fewer stations than 80% coverage at the species level.

Incidence versus abundance data

The Rock Unique data s*et al*lowed for comparison of incidence with abundance data from the grab samples. Overall, ordination patterns were similar for the two types of data as was the estimated number of stations needed to achieve a given coverage. This may be due to the counts per species being relatively 'modest', i.e. in many cases the species count did not exceed 5 or so and therefore abundance patterns would be similar to incidence patterns.

Log-transformation?

Questions were asked about whether log-transformation of the counts or biomass would be preferable. Such transformations are often used to lessen the impact of observations consisting of extremely high counts. For the Rock Unique abundance and biomass data this was not an issue (see also previous paragraph) and therefore results from log-transformed data are not presented (ordination plots were almost identical to those from the untransformed data).

Issues with data

- For some species (notably the gastropoda and polychaeta classes), there is no corresponding hierarchical specification for 'Order' in WORMS. WORMS provides the authoritative and comprehensive list of names for marine organisms and information on the taxonomic hierarchy. This makes it difficult to analyse the data to see whether trends which happen at a species or family level also apply at an Order level.

- The inclusion of taxonomic traits information as an explanatory variable in the analysis for this contract was discussed by JNCC with the contractor. Taxonomic trait information is however not currently available for many offshore species which results in many gaps. Therefore taxonomic traits information was not included as an explanatory variable.

6 References

Chao, A. (1987). Estimating the population size for capture-recapture data with unequal catchability. *Biometrics* 43, 783–791.

Faith, D.P. 1992. Conservation evaluation and phylogenetic diversity. Biological Conservation 61, 1-10

Gauch, H.G., Whittaker, R.H., & Wentworth, T.R. (1977) A comparative study of reciprocal averaging and other ordination techniques. *Journal of Ecology* 65, 157-174.

Legendre, P., & Legendre, L. (1998) *Numerical Ecology*. 2nd English Edition. Elsevier.

Shannon, C.E. (1948). "A Mathematical Theory of Communication". *Bell System Technical Journal* 27 , 379–423.

Simpson, E.H. (1949) Measurement of diversity. *Nature* 163, 688-688.

Warwick, R., & Clarke, K.R. (1995) New 'biodiversity' measures reveal a decrease in taxonomic distinctness with increasing stress. *Marine Ecology Progress Series* 129, 301-305.

7 Supplemental Tables and Figures Rock Unique data

Supplemental Table RU 1. Phylogenetic diversity (PD) for each sediment based on data identified down to the family level. PD was also calculated for each station and then averaged per sediment. Based on incidence data (including abundances treated as incidences).

			per Station	
Sediment	**# stations**	**Sediment PD γ diversity**	**Avg PD α diversity**	**As % of Sediment PD 100 - β diversity**
Coarse	12	210	72	34
Mixed	21	229	78	34
Mud	2	83	64	77
Sand	11	147	57	39
P-value[1]			0.067	

[1]P-value for effect of habitat on diversity per station, based on one-way ANOVA.

Supplemental Table RU 2. Diversity for each sediment based on abundance data identified down to the family level. Diversity was also calculated for each station and then averaged per sediment.

		Total diversity per sediment (γ diversity)			Average per station (α diversity)			Station avg as % of total (100 - β diversity)		
Sediment	**# stations**	**Shannon**	**Simpson**	**PD**	**Shannon**	**Simpson**	**PD**	**Shannon**	**Simpson**	**PD**
Coarse	12	3.67	24.8	164	2.77	11.9	59	*75*	*48*	*36*
Mixed	21	3.63	22.5	183	2.90	12.4	66	*80*	*55*	*36*
Mud	2	3.26	20.2	78	3.05	17.2	62	*94*	*85*	*79*
Sand	11	3.68	26.7	131	2.85	14.0	55	*78*	*52*	*42*
P-value[1]					0.35	0.24	0.17			

[1]P-value for effect of sediment on diversity per station, based on one-way ANOVA

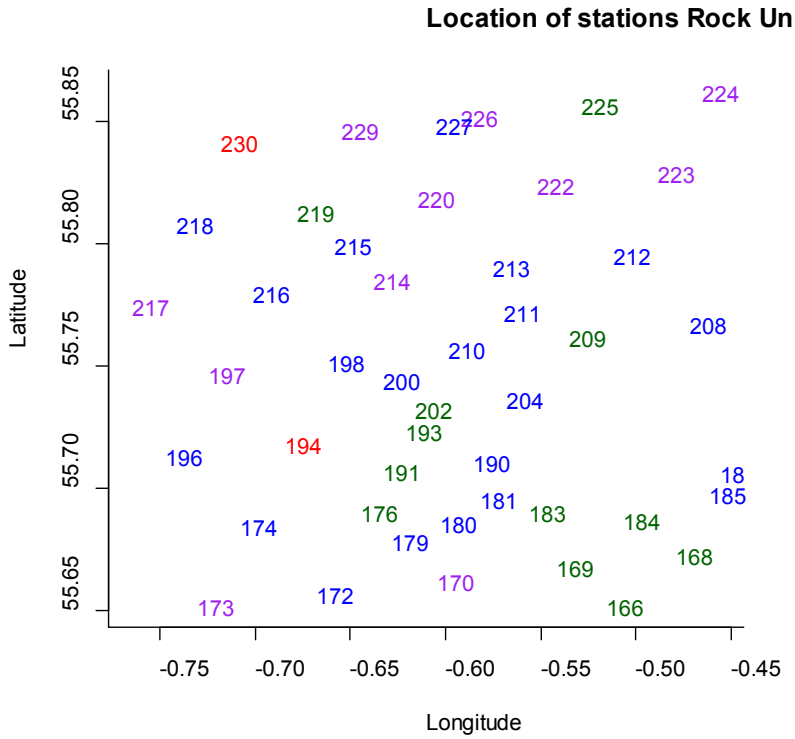
Supplemental Table RU 3. Taxonomic diversity for each sediment based on abundance data identified down to the family level. Taxonomic diversity was also calculated for each station and then averaged per sediment. Taxonomic diversity was calculated as the average distance between all possible pairs of individuals (TDavg) or pairs of species (TD01avg).

		Diversity per sediment		Average per station	
Sediment	**# stations**	**TDavg**	**TD01avg**	**TDavg**	**TD01avg**
Coarse	12	3.06	3.54	2.96[a]	3.36
Mixed	21	3.15	3.53	2.98[ab]	3.36
Mud	2	3.32	3.40	3.32[bc]	3.41
Sand	11	3.33	3.48	3.26[c]	3.39
P-value				0.004	0.931

Supplemental Table RU 4. Diversity for each sediment based on biomass data identified down to the family level. Diversity was also calculated for each station and then averaged per sediment.

		Total diversity per sediment (γ diversity)			Average per station (α diversity)			Station avg as % of total ($100 - \beta$ diversity)		
Sediment	**# stations**	**Shannon**	**Simpson**	**PD**	**Shannon**	**Simpson**	**PD**	**Shannon**	**Simpson**	**PD**
Coarse	12	2.87	8.85	155	1.69	4.21	52	*59*	*48*	*33*
Mixed	21	2.44	6.16	168	1.71	4.19	60	*70*	*68*	*36*
Mud	2	2.53	7.80	71	2.15	6.33	56	*85*	*81*	*78*
Sand	11	2.90	10.26	128	1.94	5.41	52	*67*	*53*	*40*
P-value					0.59	0.49	0.17			

[1]P-value for effect of habitat on diversity per station, based on one-way ANOVA.



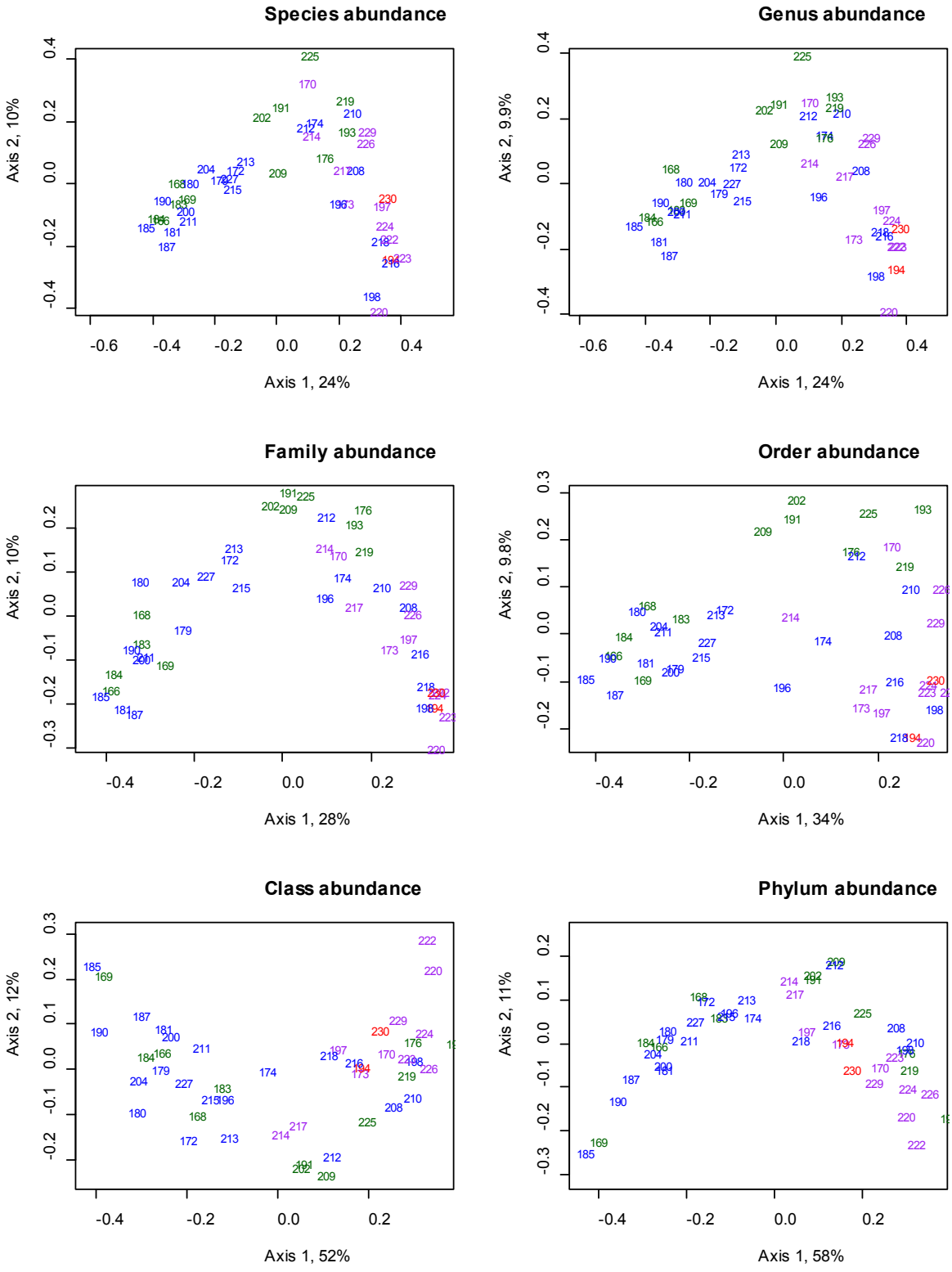
Supplemental Figure RU 1. Station locations, where each station is identified by its number. Colours indicate sediment type (red = mud, green = coarse sediment, blue = mixed sediment, purple = sand).

Station	Sediment	#species	PD	TDsum	TD01sum	Shannon	Simpson	TDavg	TD01avg
166	coarse	37	143	27765	3615	3.00	12.21	5.18	5.43
168	coarse	28	107	11230	1946	2.91	12.31	4.65	5.15
169	coarse	45	170	64576	5192	3.42	21.89	5.14	5.24
176	coarse	12	45	1914	322	2.27	8.18	4.40	4.88
183	coarse	27	112	16704	1833	2.77	9.47	5.03	5.22
184	coarse	41	150	30568	4298	3.04	11.86	4.66	5.24
191	coarse	22	88	5915	1125	2.76	11.77	4.64	4.87
193	coarse	10	43	307	223	2.21	8.00	4.65	4.96
202	coarse	27	109	5775	1806	3.07	17.53	4.91	5.15
209	coarse	17	69	2360	703	2.60	10.78	4.47	5.17
219	coarse	17	68	2360	716	2.60	10.56	5.08	5.26
225	coarse	23	88	2420	1297	3.03	18.13	5.20	5.13
172	mixed	32	118	16070	2525	3.10	15.95	4.50	5.09
174	mixed	35	133	11989	3102	3.28	19.44	4.96	5.21
179	mixed	36	138	23382	3237	3.06	12.01	4.45	5.14
180	mixed	33	123	25768	2644	3.09	16.01	4.63	5.01
181	mixed	37	139	49227	3617	2.92	10.95	5.06	5.43
185	mixed	47	170	122231	5714	3.07	12.22	5.17	5.29
187	mixed	37	141	53484	3544	2.83	9.09	5.05	5.32
190	mixed	34	123	53037	2685	3.02	14.30	4.68	4.79
196	mixed	33	125	10948	2641	3.23	17.09	4.41	5.00
198	mixed	15	57	1711	480	2.40	7.84	4.53	4.57
200	mixed	35	133	41159	3220	3.01	12.48	5.23	5.41
204	mixed	42	151	36377	4296	3.11	12.01	4.48	4.99
208	mixed	18	68	1426	708	2.74	12.52	4.39	4.63
210	mixed	15	62	1326	534	2.56	11.08	4.80	5.09
211	mixed	31	121	31919	2516	2.46	5.11	4.62	5.41
212	mixed	20	78	2624	887	2.86	15.12	4.41	4.67
213	mixed	33	129	10956	2765	3.06	12.17	4.81	5.24
215	mixed	27	106	15429	1765	2.65	7.82	4.53	5.03
216	mixed	24	95	5267	1425	2.94	14.83	4.87	5.16
218	mixed	26	98	11024	1624	2.81	11.03	4.99	5.00
227	mixed	39	142	17717	3886	3.41	24.00	5.08	5.24
194	mud	25	100	6656	1571	2.95	14.86	5.02	5.24
230	mud	23	95	4462	1363	2.93	15.21	5.18	5.39
170	sand	17	69	3052	669	2.47	8.53	4.84	4.92
173	sand	24	93	4204	1432	2.97	15.75	4.88	5.19
197	sand	30	115	6558	2292	3.09	14.54	4.95	5.27
214	sand	21	86	4864	1099	2.66	9.73	4.50	5.23
217	sand	31	114	6258	2213	3.20	18.78	4.72	4.76
220	sand	12	50	1685	331	2.23	7.36	4.80	5.02
222	sand	13	53	836	403	2.41	9.26	4.89	5.17
223	sand	18	69	2438	759	2.71	12.49	4.92	4.96
224	sand	21	81	2344	1049	2.75	10.12	5.04	5.00
226	sand	18	78	1323	839	2.77	13.56	5.23	5.48
229	sand	22	92	3472	1215	2.92	15.38	5.21	5.26

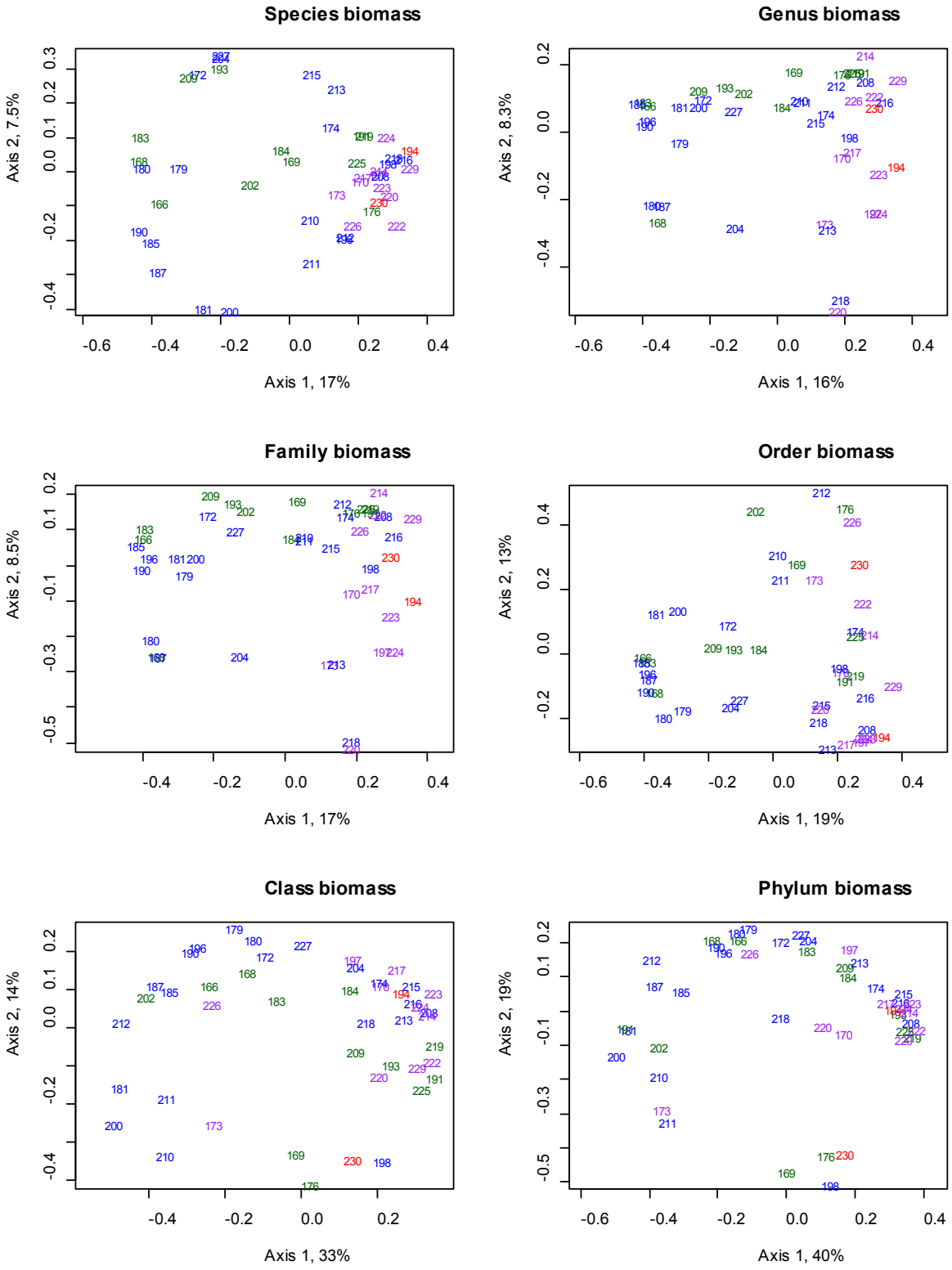
Supplemental Figure RU 2. Comparing Shannon, Simpson, phylogenetic diversity (PD) and taxonomic diversity indices. (TDsum: sum of distances between pairs of individuals or pairs of species (TD01sum); TDavg: average distance between pairs of individuals or pairs of species (TDavg)). Based on abundance data identified to the species level. For each index, the indices are coloured ranging from green for low values to red for high values.

Station	Sediment	#families	PD	TDsum	TD01sum	Shannon	Simpson	TDavg	TD01avg
166	coarse	32	73	28422	1766	2.80	9.79	3.19	3.56
168	coarse	29	64	11617	1350	2.88	11.59	2.78	3.33
169	coarse	47	97	98471	3646	3.37	20.97	2.99	3.37
176	coarse	12	32	1647	218	2.08	5.86	2.77	3.30
183	coarse	30	67	16092	1487	2.90	11.55	3.25	3.42
184	coarse	37	78	23981	2277	2.81	9.06	2.73	3.42
191	coarse	22	53	4154	701	2.71	11.00	2.80	3.03
193	coarse	13	34	460	262	2.48	10.80	3.01	3.36
202	coarse	26	63	4601	1071	2.97	15.37	2.99	3.30
209	coarse	20	51	2615	662	2.62	8.88	2.76	3.48
219	coarse	17	42	2012	457	2.63	11.57	3.19	3.36
225	coarse	23	55	3169	854	2.94	16.03	3.06	3.38
172	mixed	32	68	12668	1677	3.02	14.78	2.67	3.38
174	mixed	38	81	12743	2448	3.34	21.01	3.25	3.48
179	mixed	36	77	20835	2101	2.81	8.45	2.60	3.33
180	mixed	31	65	21422	1547	2.90	12.56	2.72	3.33
181	mixed	37	76	38273	2361	2.86	10.36	3.25	3.55
185	mixed	43	86	115236	3142	2.97	11.88	3.22	3.48
187	mixed	37	79	48152	2315	2.86	10.09	3.27	3.48
190	mixed	29	58	49504	1235	2.79	11.34	2.64	3.04
196	mixed	30	65	11137	1441	2.95	11.79	2.66	3.31
198	mixed	20	47	2292	608	2.72	11.27	3.09	3.20
200	mixed	35	73	36645	2080	3.04	14.13	3.37	3.50
204	mixed	37	80	27524	2208	2.93	10.47	2.67	3.32
208	mixed	21	45	2160	675	2.81	13.13	3.07	3.21
210	mixed	20	45	2056	623	2.83	14.41	3.09	3.28
211	mixed	31	68	27256	1651	2.54	6.05	3.11	3.55
212	mixed	22	53	3342	731	2.88	14.88	2.73	3.16
213	mixed	34	75	9785	1939	3.05	12.18	2.95	3.46
215	mixed	25	60	12804	997	2.63	7.92	2.87	3.32
216	mixed	25	60	4823	1019	2.91	13.40	3.13	3.40
218	mixed	25	59	8219	1008	2.80	11.62	3.22	3.36
227	mixed	37	74	18550	2307	3.23	18.97	3.09	3.46
194	mud	27	60	5578	1177	2.98	14.94	3.26	3.35
230	mud	27	63	4646	1215	3.12	19.37	3.37	3.46
170	sand	23	51	3692	826	2.85	12.94	3.27	3.26
173	sand	26	62	8255	1161	2.87	12.31	3.42	3.57
197	sand	31	67	7469	1597	3.14	16.17	3.28	3.43
214	sand	26	62	6463	1109	2.77	10.28	2.84	3.41
217	sand	34	66	5832	1668	3.34	23.01	2.89	2.97
220	sand	14	37	2462	313	2.36	8.59	3.32	3.44
222	sand	15	35	1582	343	2.35	7.42	3.19	3.27
223	sand	19	47	2613	577	2.71	12.45	3.19	3.37
224	sand	22	54	3254	789	2.85	13.26	3.44	3.42
226	sand	24	59	1752	1001	3.06	18.29	3.53	3.63
229	sand	26	65	4274	1131	3.09	18.94	3.49	3.48

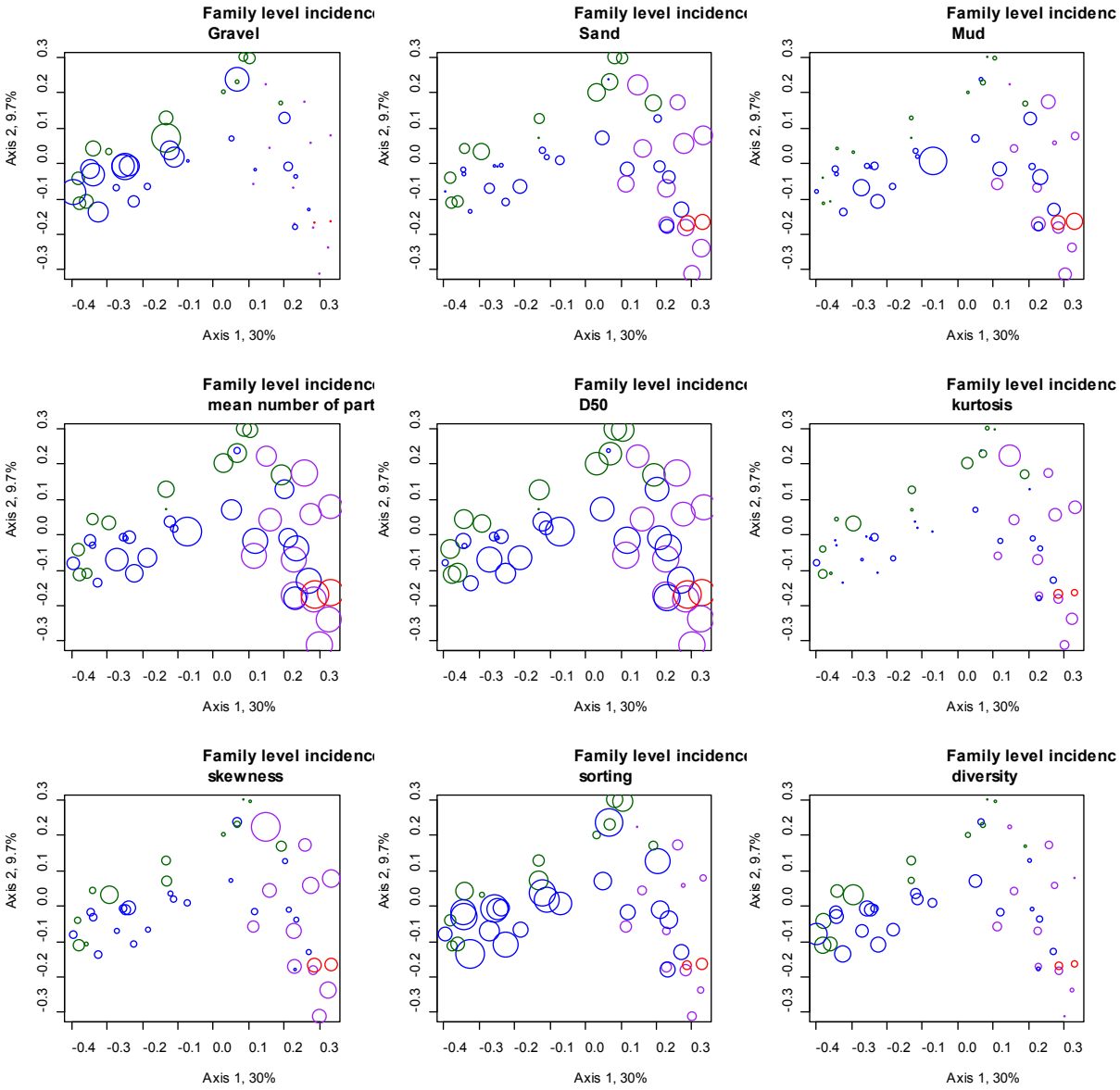
Supplemental Figure RU 3. Comparing Shannon, Simpson, phylogenetic diversity (PD) and taxonomic diversity indices (TDsum: sum of distances between pairs of individuals or pairs of species (TD01sum); TDavg: average distance between pairs of individuals or pairs of species (TDavg)). Based on abundance data identified to the family level. For each index, the indices are coloured ranging from green for low values to red for high values.



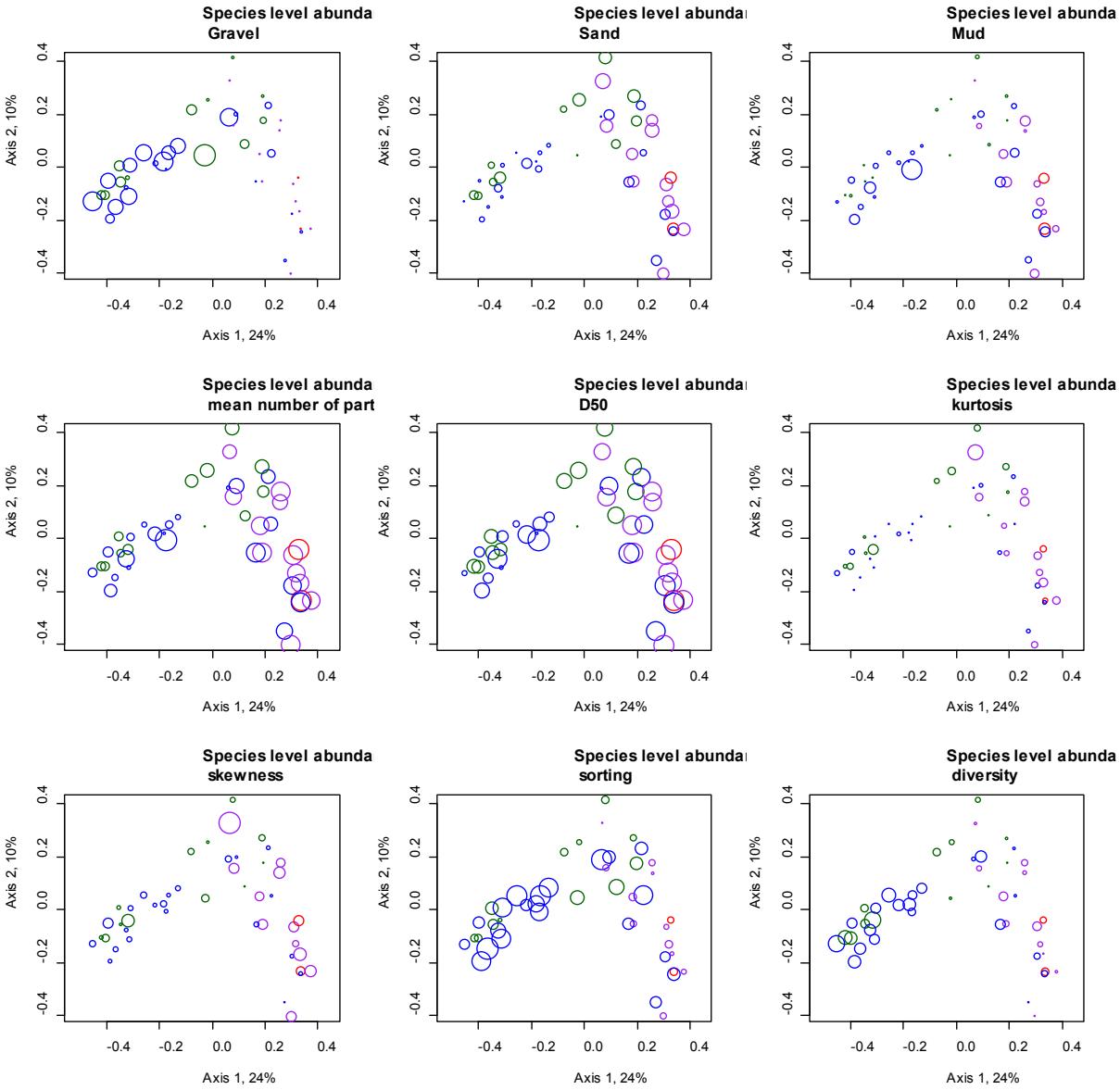
Supplemental Figure RU 4. PCO analysis using abundance data identified to species level, genus level etc. Stations are reflected by their codes and are coloured according to their sediment type (red= mud, green = coarse sediment, blue = mixed sediment, purple = sand).



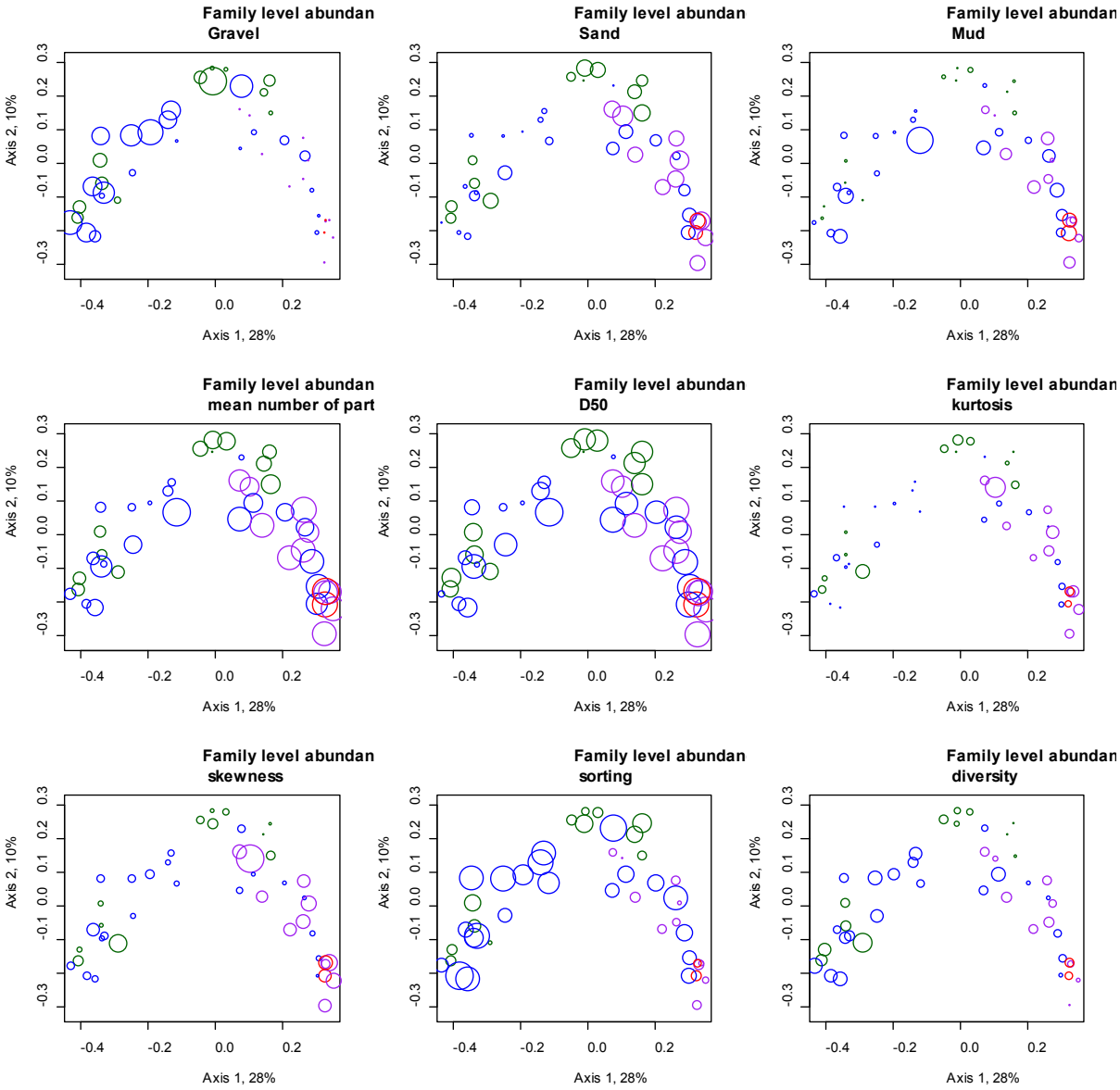
Supplemental Figure RU 5. PCO analysis using biomass data identified to species level, genus level etc. Stations are reflected by their codes and are coloured according to their sediment type (red= mud, green = coarse sediment, blue = mixed sediment, purple = sand).



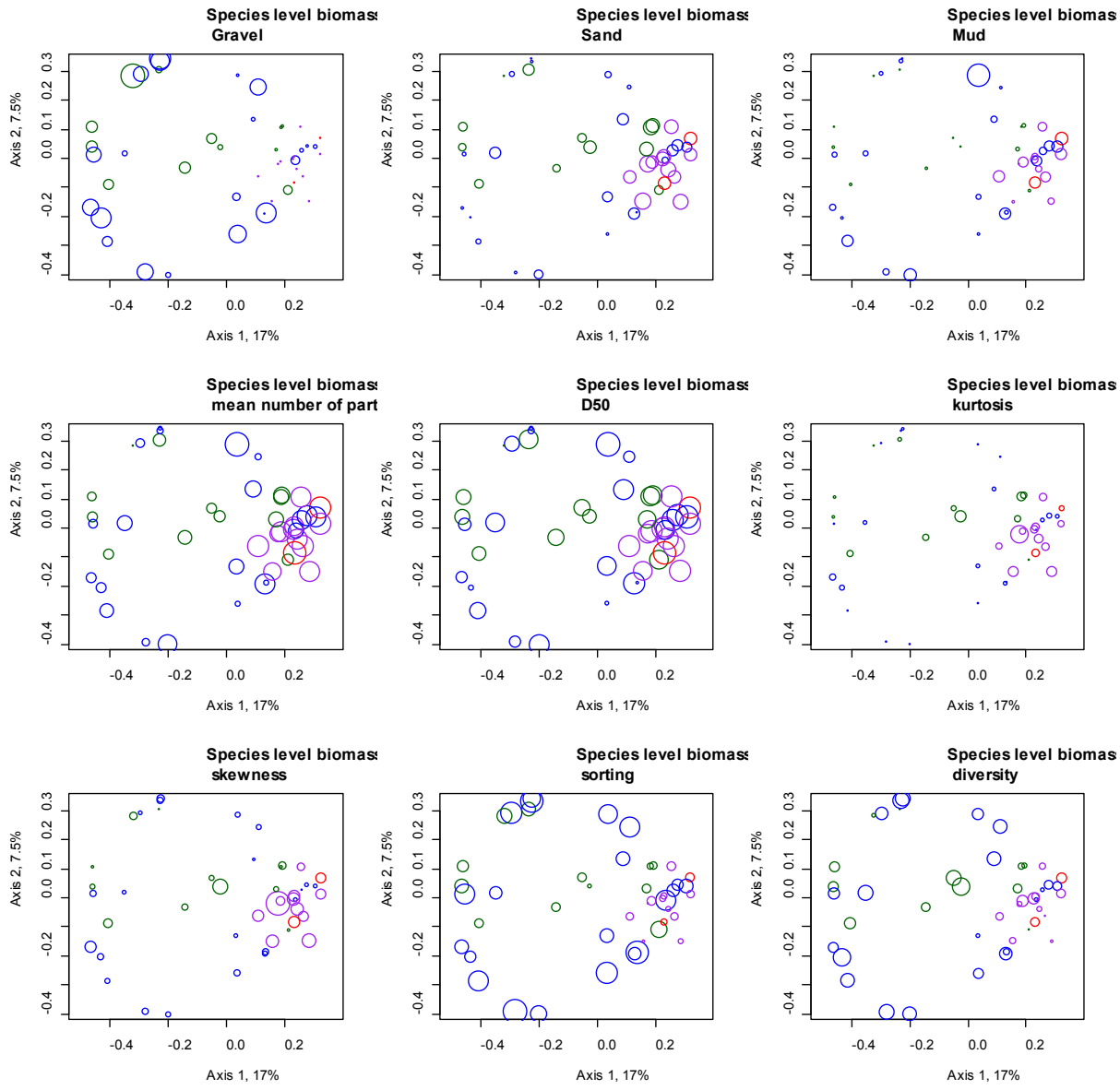
Supplemental Figure RU 6. PCO analysis using incidence data (including abundances treated as incidences) identified to at least the family level. Stations are reflected by circles, where the diameter size reflects percentage of gravel, sand, mud (first row), mean number of particles, median number of particles, kurtosis, skewness or sorting (latter five indices based on log ϕ particle size determination). The final plot shows phylogenetic diversity. The colours reflect sediment type (red= mud, green = coarse sediment, blue = mixed sediment, purple = sand).



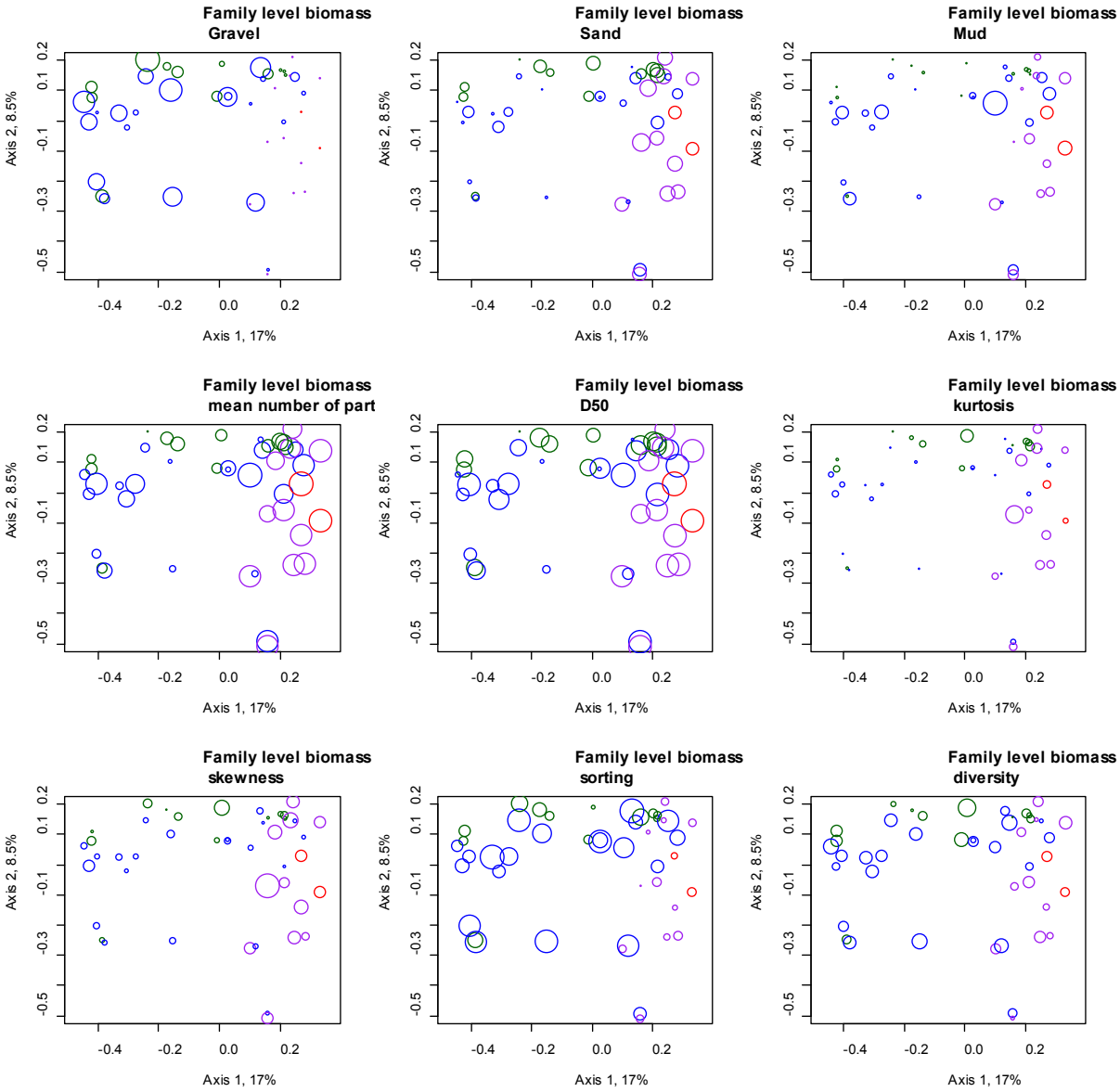
Supplemental Figure RU 7. PCO analysis using abundance data identified to the species level. Stations are reflected by circles, where the diameter size reflects percentage of gravel, sand, mud (first row), mean number of particles, median number of particles, kurtosis, skewness or sorting (latter five indices based on log ϕ particle size determination). The final plot shows phylogenetic diversity. The colours reflect sediment type (red= mud, green = coarse sediment, blue = mixed sediment, purple = sand).



Supplemental Figure RU 8. PCO analysis using abundance data identified to at least the family level. Stations are reflected by circles, where the diameter size reflects percentage of gravel, sand, mud (first row), mean number of particles, median number of particles, kurtosis, skewness or sorting (latter five indices based on log ϕ particle size determination). The final plot shows phylogenetic diversity. The colours reflect sediment type (red= mud, green = coarse sediment, blue = mixed sediment, purple = sand).



Supplemental Figure RU 9. PCO analysis using biomass data identified to the species level. Stations are reflected by circles, where the diameter size reflects percentage of gravel, sand, mud (first row), mean number of particles, median number of particles, kurtosis, skewness or sorting (latter five indices based on log ϕ particle size determination). The final plot shows phylogenetic diversity. The colours reflect sediment type (red= mud, green = coarse sediment, blue = mixed sediment, purple = sand).



Supplemental Figure RU 10. PCO analysis using biomass data identified to at least the family level. Stations are reflected by circles, where the diameter size reflects percentage of gravel, sand, mud (first row), mean number of particles, median number of particles, kurtosis, skewness or sorting (latter five indices based on log ϕ particle size determination). The final plot shows phylogenetic diversity. The colours reflect sediment type (red= mud, green = coarse sediment, blue = mixed sediment, purple = sand).

8 Supplemental Tables and Figures for Solan Bank data

Supplemental Table SB 1. Biotopes versus the codes used in the current Report.

Biotope	Code	Colour used in graphs
CR.HCR.FaT.CTub.Adig	1	Dark green
CR.HCR.XFa.SpAnVt	2	Olive green
CR.MCR.EcCr.CarSp.Bri	3	Purple
CR.MCR.EcCr.CarSp.PenPcom	4	Blue
IR.HIR.KFaR.LhypR.Pk	5	Dark blue
SS.SCS.OCS	6	Red
SS.SMx.OMx	7	Dark red
SS.SSa.OSa	8	Turquoise

Supplemental Table SB 2. Average number of families per biotope, per substation and per still.

Biotope code[1]	# Stations	# Stills	per Biotope Avg # Families	per Station Avg # Families	per Station As % of Biotope	per Still Avg # Families	per Still As % of Biotope	per Still As % of Station
1	4	8	43	23	*54*	20	*47*	*86*
2	1	2	45	45	*100*	34	*74*	*74*
3	13	49	64	31	*49*	19	*30*	*61*
4	11	39	75	41	*55*	27	*35*	*65*
5	1	1	18	18	*100*	18	*100*	*100*
6	14	30	51	12	*24*	8	*16*	*67*
7	4	7	40	17	*42*	12	*29*	*69*
8	1	2	5	5	*100*	4	*70*	*70*
Total	49	138	88	26	*29*	19	*21*	*72*

[1]See Supplemental Table SB1 for details.

Supplemental Table SB 3. Phylogenetic diversity (PD) for each biotope based on data identified to at least the family level. PD was also calculated for each station and then averaged per biotope. Likewise, PD was calculated for each still and then averaged per biotope.

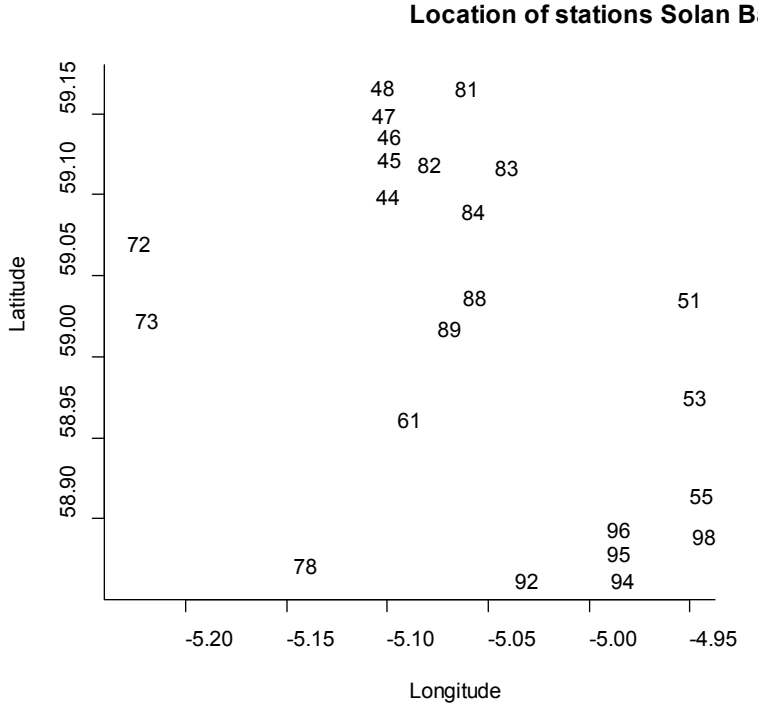
Biotope code[1]	# Stations	# Stills	Biotope PD γ div.	per Station Avg PD α div.	per Station As % of Biotope PD 100 - β div.	per Still Avg PD	per Still As % of Biotope PD	per Still As % of Station PD
1	4	8	101	64	*63*	57	*56*	*89*
2	1	2	94	94	*100*	76	*80*	*80*
3	13	49	126	74	*59*	51	*40*	*69*
4	11	39	140	89	*64*	64	*46*	*72*
5	1	1	59	59	*100*	59	*100*	*100*
6	14	30	109	35	*32*	25	*23*	*71*
7	4	7	91	44	*48*	31	*34*	*71*
8	1	2	14	14	*100*	11	*79*	*79*
Combined	49	138	164	62	*38*	48	*29*	*78*

[1]See Supplemental Table SB1 for details.

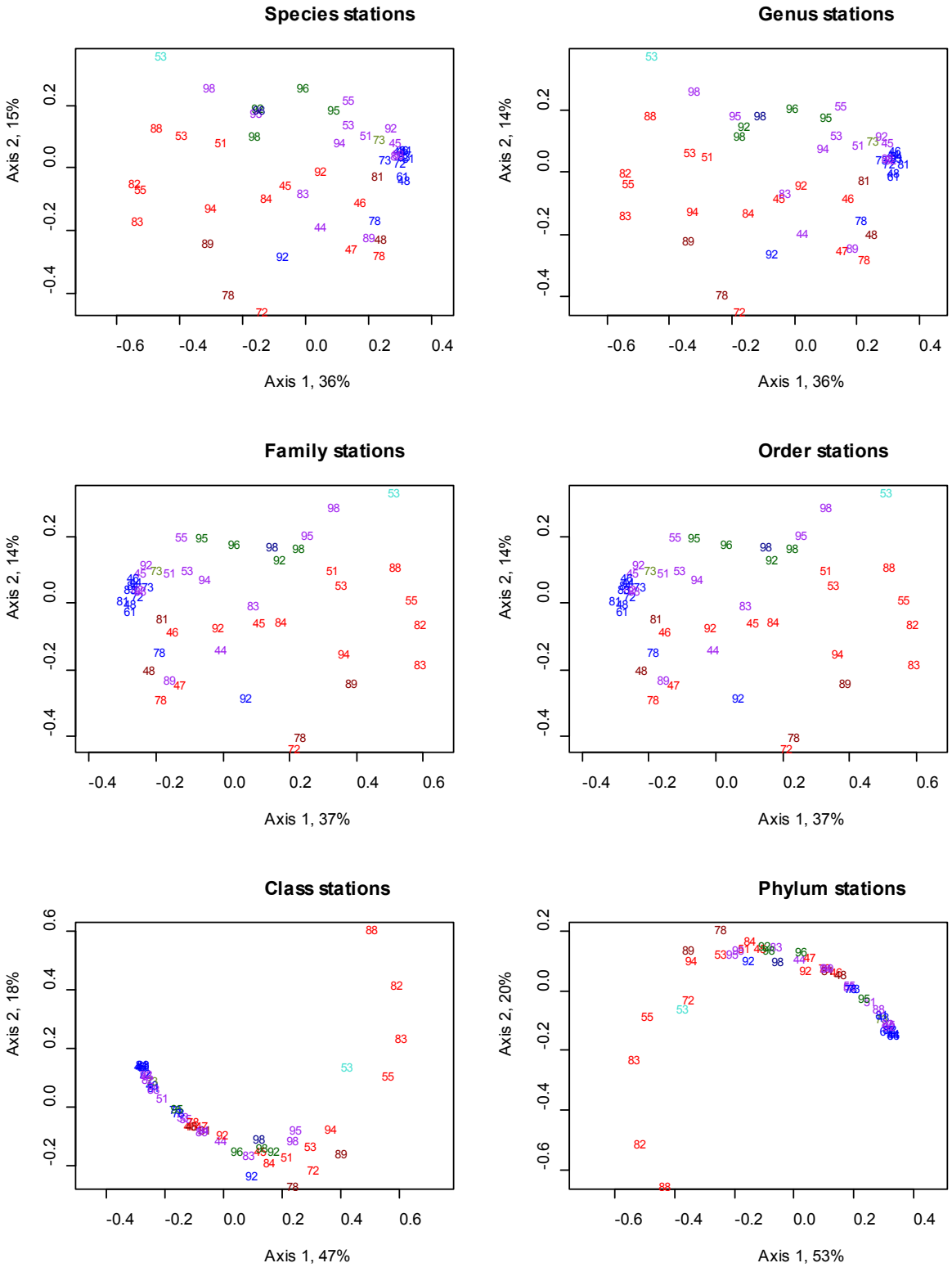
Supplemental Table SB 4. Taxonomic diversity expressed as average distance between pairs of families (TD01avg) for each biotope based on data identified down to the family level. PD was also calculated for each station and then averaged per biotope. Likewise, PD was calculated for each still and then averaged per biotope.

Biotope	Code[1]	# Stations	# Stills	TD Biotope	Avg TD per Station	Avg TD per Still
CR.HCR.FaT.CTub.Adig	1	4	8	4.01	3.92	3.90
CR.HCR.XFa.SpAnVt	2	1	2	3.74	3.74	3.72
CR.MCR.EcCr.CarSp.Bri	3	13	49	3.79	3.82	3.84
CR.MCR.EcCr.CarSp.PenPcom	4	11	39	3.78	3.75	3.75
IR.HIR.KFaR.LhypR.Pk	5	1	1	4.24	4.24	4.24
SS.SCS.OCS	6	14	30	3.82	3.88	3.71
SS.SMx.OMx	7	4	7	3.81	3.79	3.76
SS.SSa.OSa	8	1	2	3.30	3.30	3.65
Combined		49	138	3.90	3.82	3.79

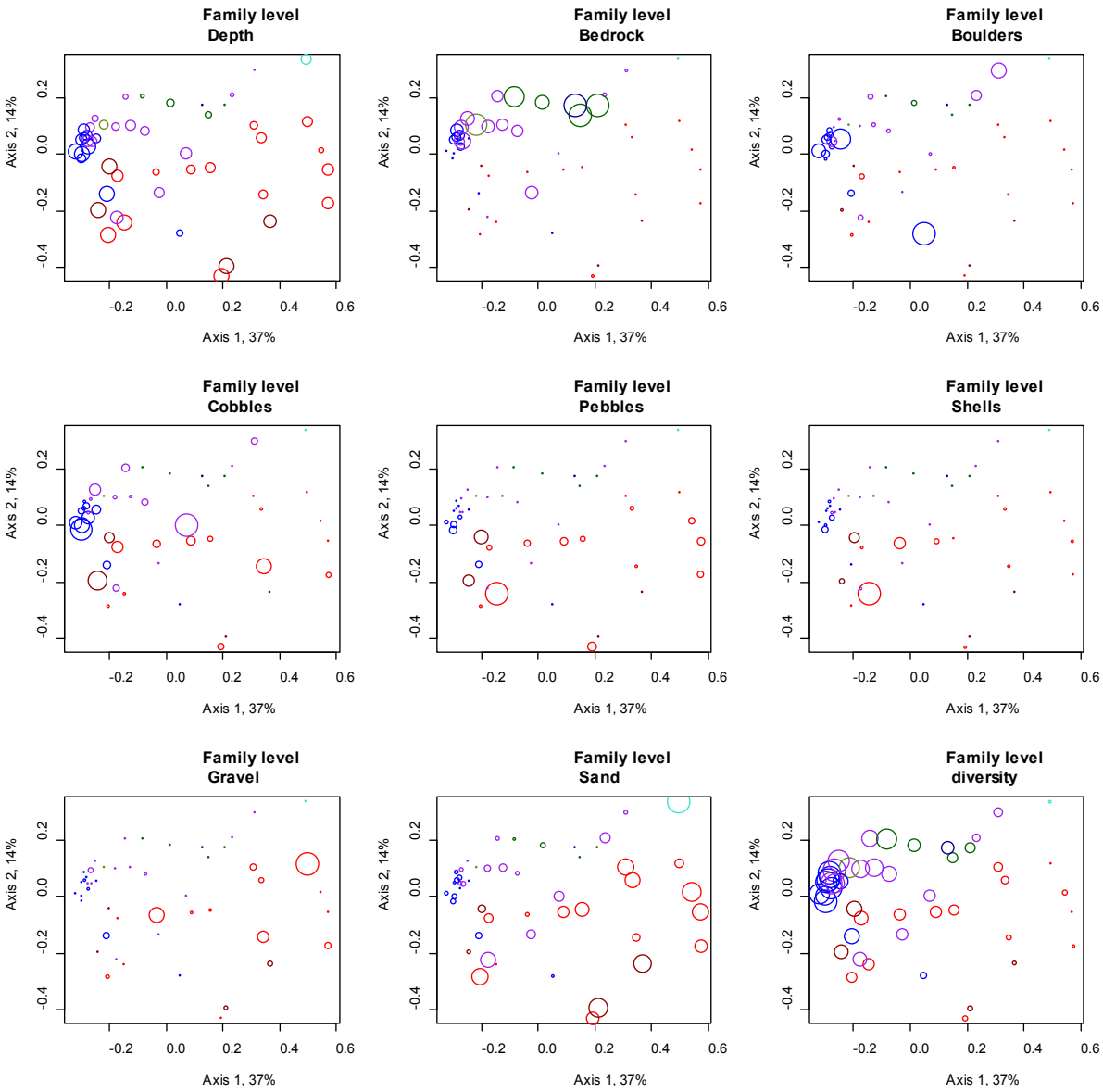
[1]See Supplemental Table SB1 for details.



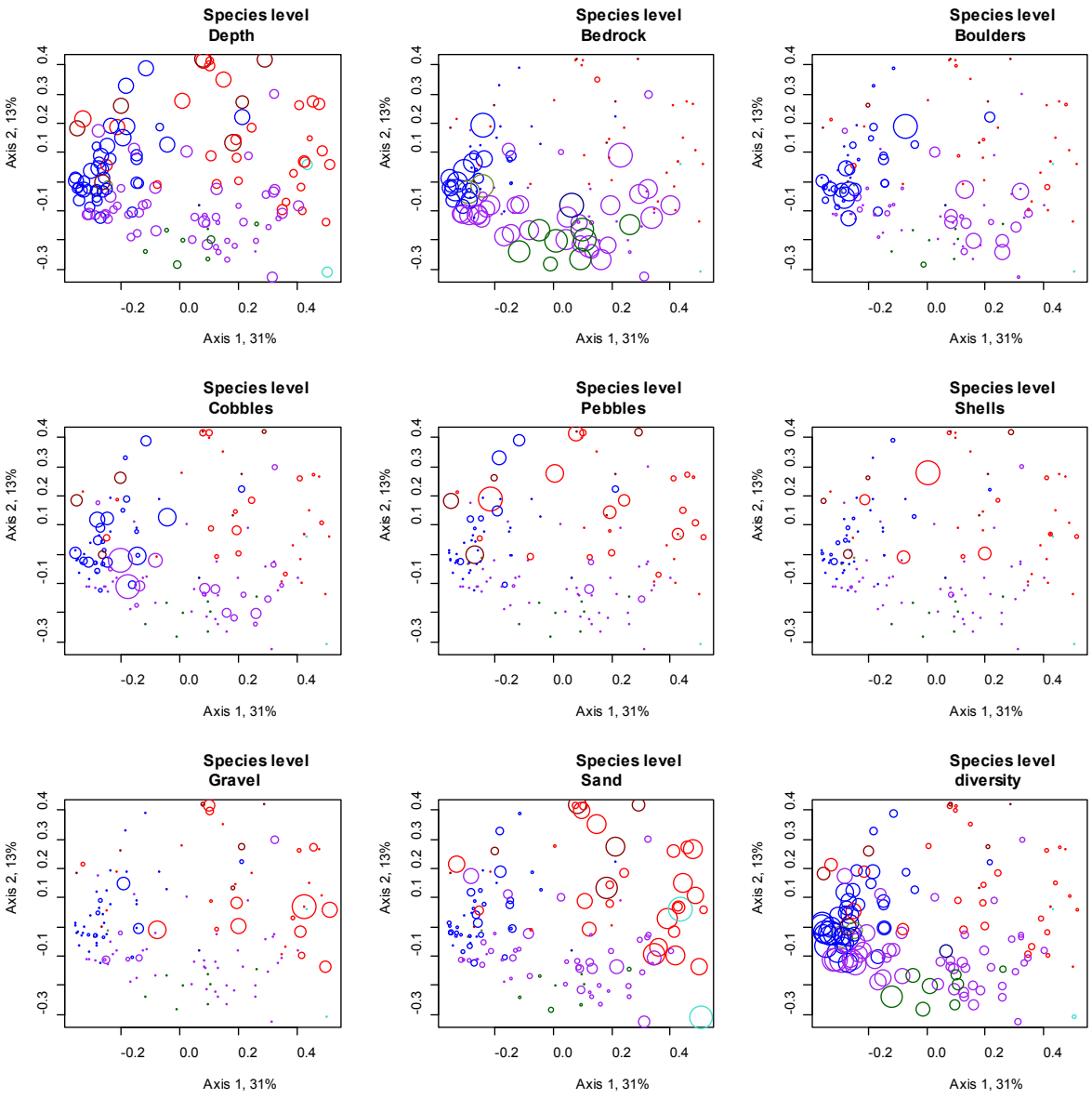
Supplemental Figure SB 1. Station locations, where each station is identified by its number.



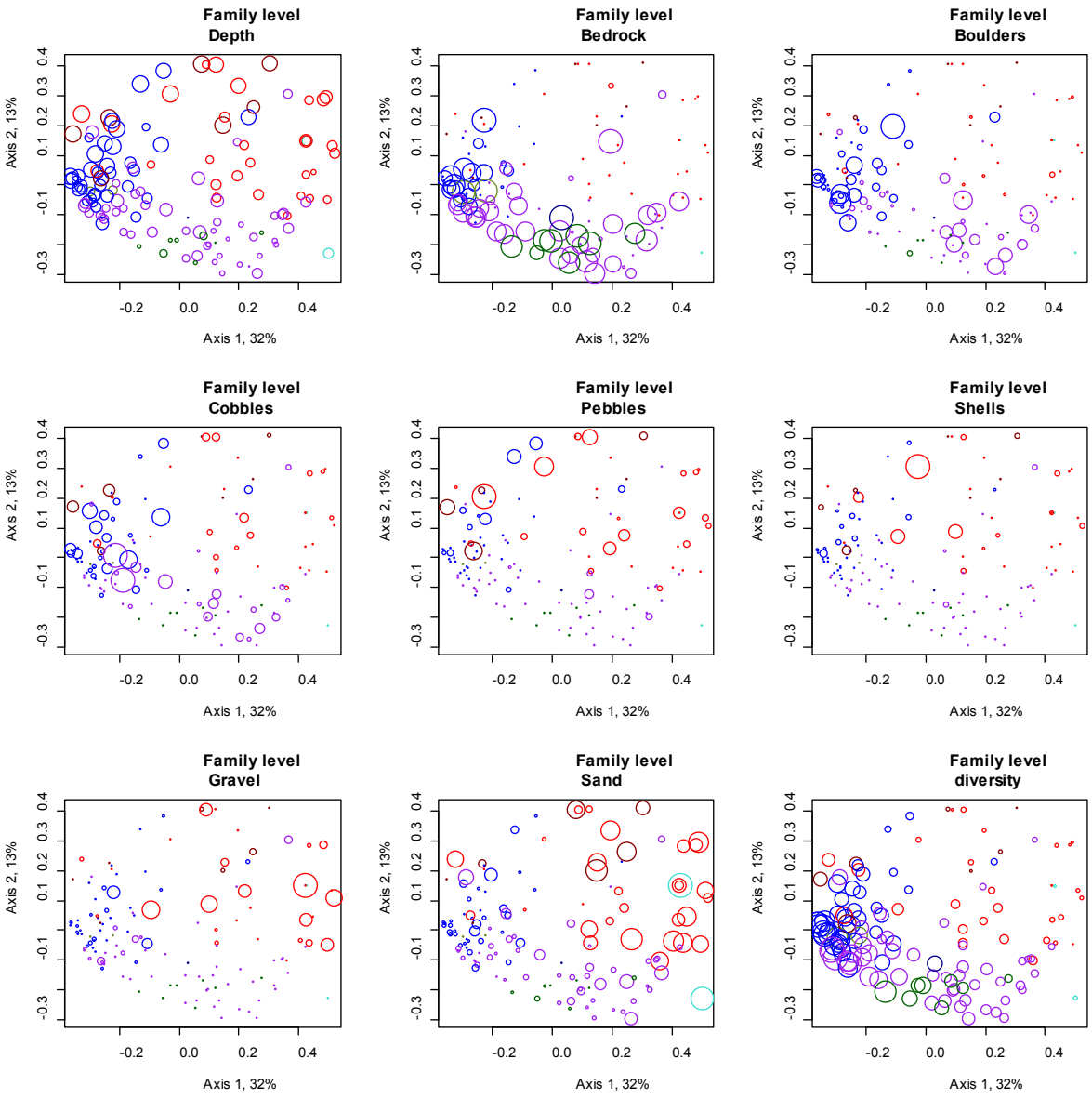
Supplemental Figure SB 2. Principal coordinates analysis for incidence data observed that each substation.Each substation is be presented by its corresponding station number and colours reflect the different biotopes (see Supplemental Table SB1). As several stations cover more than one biotope, station codes can appear more than once. Each graph is based on all observations that were identified to at least the corresponding phylogenetic level in its title.



Supplemental Figure SB 3. The ordination species data per substation is shown here, with each substation represented by a circle. The diameter is an indication of depth, percentage bedrock etc. The final plot shows phylogenetic diversity. Colours reflect biotopes (see Supplemental Table SB1). Based on data identified to at least the family level.



Supplemental Figure SB 4. The ordination species data per still is shown here, with each still represented by a circle. The diameter is an indication of depth, percentage bedrock etc. The final plot shows phylogenetic diversity. Colours reflect biotopes (see Supplemental Table SB1). Based on data identified to species.



Supplemental Figure SB 5. The ordination family data per still is shown here, with each still represented by a circle. The diameter is an indication of depth, percentage bedrock etc. The final plot shows phylogenetic diversity. Colours reflect biotopes (see Supplemental Table SB1). Based on data identified to at least the family level.