NATURE METRICS
DNA-BASED MONITORING

Phase 2 Technical Appendices - Developing Habitat Scale DNA Monitoring in Support of Post 2020 Biodiversity Reporting Requirements

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PROJECT FACILITATION

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[§]Project Advisory Board Member

Contents

1 Preface

The Phase 2 Technical Appendices presented here contains supplementary information, such as additional data analyses that were either not essential or too lengthy to be included in the core deliverable (Phase 2 Main Report) output. This includes extra data that has been analysed and underpins the results/discussion (whether they are fruitful or otherwise) but has not been provided upfront in the Phase 2 Main Report. This is primarily for more specialist technical and scientific experts as well as anyone interested in further evidence underpinning the other core Phase 2 project deliverable outputs. For outputs such as Operational Taxonomic Units (OTU) tables and Gapfinders, these have been producedd as separate excel files and referred to in this document.

2 Executive Summary

Biodiversity loss is widely recognised as one of the most urgent global challenges to be addressed in the next decade. The Scottish Biodiversity Strategy sets out a clear ambition to be Nature Positive by 2030, and to have restored and regenerated biodiversity across the country by 2045 (Scottish Government 2022). To protect, restore, and regenerate biodiversity, it is necessary to be able to accurately describe and quantify ecological change. Biodiversity monitoring through environmental DNA (eDNA) analysis is increasingly being used for tracking species diversity and composition in ecosystems as it is a scalable and high-resolution method. The overall goal of this project was to investigate and test the applicability of eDNA-based monitoring approaches for biodiversity assessment and reporting purposes across a broad range of habitat types in Scotland.

Samples were collected across four habitat types: marine lochs, freshwater lochs, woodland, and peatland. The survey sites were mostly situated in and around the focal study area of Loch Lomond and the Trossachs National Park (LLTNP) but eDNA sampling included other parts of Scotland such as the Cairngorms National Park. This was a Proof-of-Concept study across small numbers of sites and gradients of condition across Scotland. This work was undertaken to help establish scientific evidence, blended with practical learning-by-doing experience, and provide key recommendations, including future perspectives, to inform the development and implementation of eDNA-based habitat monitoring programmes for Scotland going forward. Throughout this document we use the term ‘eDNA-based’ to encompass all DNA collected from environmental substrates, which includes both extracellular DNA and whole organisms such as soil fauna samples and microeukaryotes in marine sediments (Pawlowski et al. 2020).

Across the four surveyed habitat types we found that eDNA-based data can detect compositional shifts in species communities that are associated with ecosystem state or habitat classification (freshwater: loch Water Framework Directive Overall Status, marine: biotope, woodland: restoration/regeneration class, peatland: restoration class). Using Random Forest algorithms, the eDNA-based data can be used to classify sites according to ecosystem state or restoration gradient class. These findings were most evident for the

freshwater and woodland habitats. While the data for the marine and peatland habitats were not sufficient for classification.

Numerous species with important biodiversity monitoring designations can also be detected including SSSI[1]-listed species, IUCN[2] threatened species, PMF[3] and invasive species (species whose introduction or spread threatens biological diversity).

In some cases, eDNA-based data can likely be fed directly into existing community-based indicator metrics. For example, marine sediment health scoring categories[4] were comparable to those calculated from morphological surveys and freshwater loch chironomid scoring produced similar values to those produced using best-matching conventional (CPET) data[5]. However, this approach underuses much of the data and alignment of eDNA-based data into existing models can produce differing results and might consequently not be accepted. eDNA-based data should primarily be viewed as a 'new' tool, with new models, not necessarily as a tool to shoehorn into existing indices (with exceptions).

Developing national eDNA-based datasets to operationalise these findings will require well-considered site choices along well-defined gradients that are of highest priority for meeting monitoring and reporting needs. The breadth of potential applications is large. Successful future development and implementation will depend on posing targeted biomonitoring questions for specific objectives within national and international reporting frameworks.

The specific Key Recommendations from this project are:

- For freshwater lochs, build a national ecosystem-state prediction tool based on the methods presented in the project. This would be a scalable and efficient method for tracking loch quality state and change. It will require multiple lochs across a wide geographic range.
- As part of the ecosystem-state prediction tool, conduct a validation study for chironomid scoring by conducting side-by-side studies with conventional methods (CPET).
- For marine monitoring of vertebrate PMF species, develop standard monitoring guidance using aquatic eDNA sampling.
- For marine sediment health scoring, validate further at sites with greater pollution gradients.
- For marine biotope classification, conduct further research into optimal eDNA assays for maximised indicator species detection.

[1] Sites of Special Scientific Interest
[2] International Union for Conservation of Nature
[3] Priority Marine Feature
[4] Using the AZTI Marine Biotic Index (AMBI)
[5] Using the Water Framework Directive Chironomid Pupal Exuvial Technique (CPET)

- For woodland, and other terrestrial habitats which use fungi as part of SSSI selection, further validate eDNA-based approaches for detection of SSSI-listed fungal species.
- For woodland restoration/regeneration monitoring, we initially recommend using eDNA-based data at the site level to monitor programme progress. In the longer term, a national eDNA-based survey across multiple woodland types in Scotland could be used as input to a systematic conservation planning exercise to rank woodlands by conservation value, and the higher-value woodlands can then be used as restoration targets.
- For peatland, there was a clear difference between degraded and restored peatlands, but the classification model was unable to predict status, due to the small size of this dataset. Classification of restoration status from eDNA-based data will require a large training dataset with a suitable sampling design and clear status definitions.

The Key Knowledge Gaps & Barriers are:

- Using eDNA-based data for biomonitoring at a national level in a regulatory context requires ecological frameworks based on national baselines, such as Ecological Quality Ratio (EQR) models. There are currently very few such frameworks based on or incorporating eDNA data (the Lake Fish Classification Index being the exception). Developing such frameworks requires large scale studies with focussed objectives. Biomonitoring at local scales is already possible through careful study design.
- The number of samples required for biomonitoring at the national level using eDNA-based data remains largely unanswered. This is partially due to fact that the breadth of potential applications is large, spanning numerous taxonomic groups, habitats, and biomonitoring objectives. Identifying the number of samples required for each specific biomonitoring objective is required.
- There are numerous eDNA-based projects being carried out in Scotland at various scales and in various contexts of biomonitoring, yet the data is not being captured in a systematic and unified way. Standardised guidance for formatting and storing eDNA-based data in publicly available databases would allow research in this area to progress faster. Systems such as the European Nucleotide Archive provide platforms for that could be used to store and access eDNA-based data for biomonitoring.
- There remains opportunity to develop minimum standards and validation scales to ensure consistency across projects and providers.

In most cases, eDNA-based approaches can be used to classify sites along ecological gradients. Until larger ecological biomonitoring frameworks for eDNA-based data are developed, eDNA-based approaches for national level reporting will likely remain underutilised. In the meantime, practitioners are using them for efficient surveying of key taxonomic groups. Local and regional projects are already using eDNA-based approaches to monitor negative and positive impacts of land management and restoration. The true power of eDNA-based data lies in the ability to

generate huge datasets that can be used build national-level models of biodiversity and characterise ecological conditions for robust and consistent monitoring and reporting purposes.

Overall, eDNA-based approaches can provide the necessary scaling up of biodiversity monitoring for a national monitoring strategy across multiple habitat types, increasing the number of samples that can realistically be collected and analysed, and improve the reporting efficiency through standardised field and laboratory methodologies and data formats.

3 Assay Details, Target and Non-target Species

For all data analyses in this project, only target Operational Taxonomic Units (OTUs) were used. These are OTUs belonging to taxa that are targeted by the selected assay, for example, for the fish assay, only OTUs identified as fish were utilised (with the exception of reporting marine mammal PMF species). It should also be noted that OTUs that could not be assigned to at least Kingdom level were excluded.

Table 1: Summary of the assays used; target taxa; non-target taxa; gene; forward primer sequence; reverse primer sequence and references.

Assay	Target	Non-Target	Gene	Fwd sequence	Rev sequence	Reference
Vertebrates	Chordates	n/a	12S	ACTGGGATTAGATACCCC	TAGAACAGGCTCCTCTAG	(Riaz et al. 2011; Kelly et al. 2014)
Fish	All fish	Non-fish chordates (e.g., mammals)	12S	GYYGGTAAAMYTCGTGCCAGC	CATAGYGGGGTATCTAATCCCRGTTTG	(Miya et al. 2015)
Freshwater insects/invertebrates	All non-chordate animals	Chordates	COI	GGDACWGGWTGAACWGTWTAYCCHCC	CAAACAAATARDGGTATTCGDTY	(Leese et al. 2021)
Soil invertebrates	All non-chordate animals	Chordates	18S	GGWACWRGWTGRACWITITAYCCYCC	TANACYTCNGGRTGNCCRAARAAYCA	(Capra et al. 2016)
Marine sediment invertebrates	All non-chordate animals	Chordates	COI	GGWACWGGWTGAACWGTWTAYCCYCC	TANACYTCNGGRTGNCCRAARAAYCA	(Leray et al. 2013)
Marine sediment bacteria	Bacteria	Archaea	16S	GTGYCAGCMGCCGCGGTAA	GGACTACNVGGGTWTCTAAT	(Caporaso et al. 2011)
Soil bacteria	Bacteria	Archaea	16S	GTGYCAGCMGCCGCGGTAA	GGACTACNVGGGTWTCTAAT	(Caporaso et al. 2011)
Soil fungi	Fungi	n/a	ITS2	GCATCGATGAAGAACGCAGC	TCCTCCGCTTATTGATATGC	(White et al. 1990)
Marine sediment eukaryotes	Eukaryotes	n/a	18S	CCCTGCCHTTTGTACACAC	CCTTCYGCAGGTTCACCTAC	(Amaral-Zettler et al. 2009)

4 Sampling Plan Overview

A brief outline is given here - for full details, please see the Phase 1 Pilot Study Findings & Phase 2 Sampling Plan (Egeter et al. 2023).

4.1 Marine

Based on the results of the pilot study, while Loch Goil is part of the Upper Loch Fyne and Loch Goil MPA, water and sediment samples were collected by The Marine Directorate (formerly Marine Scotland Science, MSS) predominantly from Loch Long to mitigate the effects of freshwater input. Four different sediment-based biotopes in Loch Long were selected for sample collection.

The following key sources of best available data were used to inform sampling design for the marine habitat:

- Moore 2013; NatureScot Commissioned Report 631: Biological analyses of underwater video from research cruises in the Clyde Sea (Loch Goil and the south of Arran) and in Orkney (Rousay Sound and Stronsay Firth)
- Allen et al. 2013; SNH Commissioned Report 437: Marine biological survey to establish the distribution of Priority Marine Features within the Clyde Sea area
- Consultation with key stakeholders from Scottish Government agencies

4.2 Freshwater

Following the pilot study, freshwater lochs became the focus of the freshwater habitat Phase 2 eDNA survey. The total number of sites, sampling locations, and sample assays was initially decided based on balancing the project budget and resources available, with obtaining the range of sites and level of replication required to address whether eDNA metabarcoding can enable assessment of habitat condition of Scottish freshwater lochs. However, the decisions regarding exactly how many reasonably representative samples to collect and at which freshwater lochs sites to sample, beyond Loch Lomond, required extensive consideration and consultation with key project stakeholders with relevant technical expertise and practical experience of operationalising monitoring resources across Scotland.

The collection of 10 shoreline samples in winter was previously identified as the minimum sampling effort required to detect ≥85% of fish species present in UK lakes (Li et al. 2019). However, this level of sampling effort may or may not be achievable for freshwater lochs if eDNA shoreline monitoring approaches were upscaled in the future.

It was decided that six samples per freshwater loch were to be collected from the shoreline. This approach was standardised across all freshwater lochs sampled for the Phase 2 eDNA survey. This fixed sample number was considered the reasonable balance between the minimum required for DNA-based sampling, loch accessibility reasons (not all parts of the sampled lochs were accessible by land), and available contractor resources (budgetary constraints) to deliver the project work in 2022. By taking that key decision, it was possible to

increase the total number of freshwater lochs that could be sampled for metabarcoding analysis from the shoreline, and in doing so expand breadth of the overall habitat pressure gradient assessed.

Following extensive consideration and consultation with key project stakeholders, including experts from SEPA and NatureScot, a total of 15 freshwater lochs were selected based on their location within, or their proximity to LLTNP, accessibility by road, and where shoreline sampling would be sufficient (to minimise resource constraints, also keeping in mind potential future monitoring programmes). All 15 lochs had previously been classified by SEPA using the WFD 'overall status' designations and we chose them against the criteria specified that could be met for high, good, moderate, and poor classification status.

It was important that the lochs were reasonably reflective of WFD overall status as high, good, moderate, and poor or bad ecological status, whilst also ensuring the overall hydrology status remained high so that impacts from major known confounding factors (such as hydrology pressure from impoundment or abstraction due to hydropower or water supplies) were reasonably minimised wherever feasible, especially if any Scottish lochs are designated as Heavily Modified Waterbodies (HMWBs) and Grouped Waterbodies. We selected lochs that were:

- Lowland situated (Altitude type <200 m according to WFD-UTKAG, 2004)
- Have a large surface area (size type ≥ 0.5 km^2 in surface area according to WFD-UTKAG, 2004)
- Situated within a reasonably similar geographic area and climatic envelope, with most lake sampling constrained to the LLTNP focal study area, with some acceptable distances up to a 100 km radius beyond LLTNP boundaries
- Reasonably representative of standing waterbodies located within the focal study area of LLTNP:
    - Mostly low alkalinity, with some acceptable and occasional deviation into moderate alkalinity (according to WFD-UKTAG, 2014)
    - A balanced mixture of deep and shallow waterbody depth types, with 'very shallow' being the occasional exception (according to WFD-UKTAG, 2014)
    - Mostly clear water colour types, with some acceptable and occasional deviation into humic, polyhumic, or unknown types (according to WFD-UKTAG, 2014)
- Reasonably representative of a range of land use categories including moorland, arable, woodland, and urban land cover in the surrounding catchments
- There is recent evidence that some freshwater lochs are impacted by climate change (May et al. 2022). It was found that Loch Achray and Loch Lubnaig situated in LLTNP to be amongst the most rapid warming standing waters in Scotland, with water temperatures having increased by between 1.0 and 1.3°C per year during 2015-2019)

The following key sources of best available data were used to inform sampling design for the freshwater habitat:

- Water Classification Hub (sepa.org.uk)
- UK Lakes Portal (ceh.ac.uk)

- wfd uktag | water framework directive e.g.,
    - WFD-UKTAG (2004) Guidance on Typology for Lakes for the UK | wfd uktag
    - WFD-UKTAG (2014) UKTAG Lake Assessment Methods (wfduk.org)
- Assessing climate change impacts on the water quality of Scottish standing waters | CREW | Scotland's Centre of Expertise for Waters
- https://www.space-intelligence.com/scotland-landcover/
- https://www.nature.scot/professional-advice/protected-areas-and-species/protected-areas
- Lochwinnoch Nature Reserve, Renfrewshire, Scotland - The RSPB
- Consultation with key stakeholders from Scottish Government organisations

4.3 Woodland

Within the scope of the project objectives, we aimed to assess whether eDNA communities and derived metrics can indicate overall woodland condition across a restoration gradient, from unforested, recently planted/reforested, and mature Scots pine woodland habitats. We used eDNA metabarcoding data to track woodland restoration of Scots pine at different stages of regeneration. This means that chronosequences (the different stages of regeneration) of restored woodland were used as a proxy for monitoring over time.

It was decided to focus on restoration gradient in Caledonian pine forest, Scots pine (*Pinus sylvestris*). This increased our chances of obtaining clear, unequivocal results, which is a common aspiration across all key stakeholders. Moreover, using Scots pine has the benefit of tying into pre-existing and parallel work by Forest Research. Although these Forest Research experimental Scots pine sites are not within LLTNP, the setup of the sites warranted sufficient merit to include in this study. Because not all sites had all three categories, one of the Cairngorms Forest Research sites, Rothiemurchus, situated within the Cairngorms National Park, was chosen to be included in the main sampling campaign of this project. Moreover, Rothiemurchus had their own adjacent young and natural regeneration mature Scots pine, which made for a better comparison.

By using monoculture stands of Scots pine at different stages of regeneration, space was substituted for time by using chronosequences of restoration. Three chronosequence categories were chosen instead of four to obtain better replication per treatment; unforested, recently planted/reforested, and mature condition. All sites were required to contain all the chosen age categories. Within each site, the different categories were required to be the same forest type, i.e. Scots pine. To further exclude confounding factors, the different categories were also required to be in similar environments, e.g. we did not want to compare areas on a steep slope or high plateau with lochside areas. Ideally, the sites needed to have each of the categories in adjacent stands, or at least in close proximity to each other.

Categories were a chronosequence of forest age. Three categories were selected; unforested (which may range from grassland to moorland), recently planted/reforested, and mature condition. Unforested areas are representative of an area that would be forest if it wasn't grazed (such as grassland or moorland). Mature condition forest is the target, while recently

planted/reforested is "regenerating" forest on its way to target status. The sampling locations and their respective categories were chosen based on the above and on extensive consultation with all key stakeholders. Two of the woodland sites selected for Phase 2 eDNA sampling were situated within the LLTNP and are both SSSI (Coille Coire Chuilc and Glen Falloch), while Rothiemurchus sits within the Cairngorms National Park. Coille Ruigh and Ghubhais are both SSSI and SAC areas. Those sites which did not fall within Scotland's designated site network functioned to provide replicates for the chronosequences established for the woodland sites.

4.4 Peatland

Within the scope of the project objectives we aimed to test whether peatland sites of differing condition categories (degraded or restored) have different biological communities that can indicate overall condition using eDNA metabarcoding.

Site selection criteria required sites with varying peat condition - degraded and restored. Originally a third category (unimpacted) was proposed. However, because 70% of Scotland's blanket bog and 90% of Scotland's raised bog peatland is degraded (Artz et al. 2014), as such, the Peatland ACTION officer was unable to suggest any good/unimpacted condition peatland within LLTNP. Furthermore, despite searching while on site, no patches of good/unimpacted condition peatland were identified at any of the sites. Accordingly, it was not possible to find unimpacted areas to include in this study. Site selection was then based on two categories.

Based on the criteria three sites were selected. Glen Finglas, Auchlyne, and Cashel. Glen Finglas and Auchlyne contain drained and restored (through grip blocking) peatland. The Cashel site covers a large area on the south-east side of LLTNP but did not contain any areas that were not drained. However, restoration work is expected to start in 2023. When selecting damaged/drained areas this should be based on locations that are likely to go forward for restoration as this will allow future restoration time series assessments to be made.

Sampling locations were based on the following criteria:

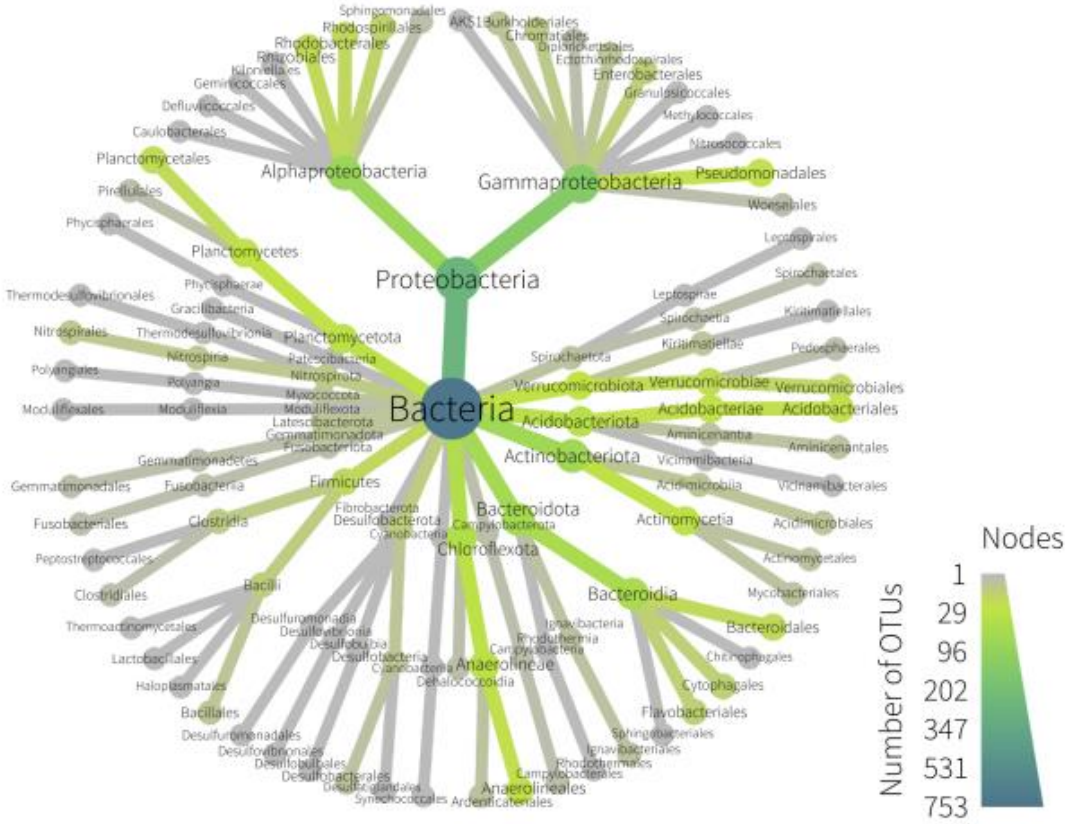
- Approximate density of sampling points at 2 per km^2
- Within areas of known peat (e.g. using PEATMAP; (Xu et al. 2018); or Carbon and peatland 2016 map when available) and with varying condition between restored and degraded peat
- Within approximately 2 km of a road to allow accessibility
- Sample locations within Glen Finglas and Auchlyne were selected because these are upland blanket bog sites within LLTNP where restoration works have been undertaken as part of the Peatland ACTION project.
- Sampling locations were determined on site in consultation with a Peatland ACTION representative and site managers

5 Overview of Taxa Detected

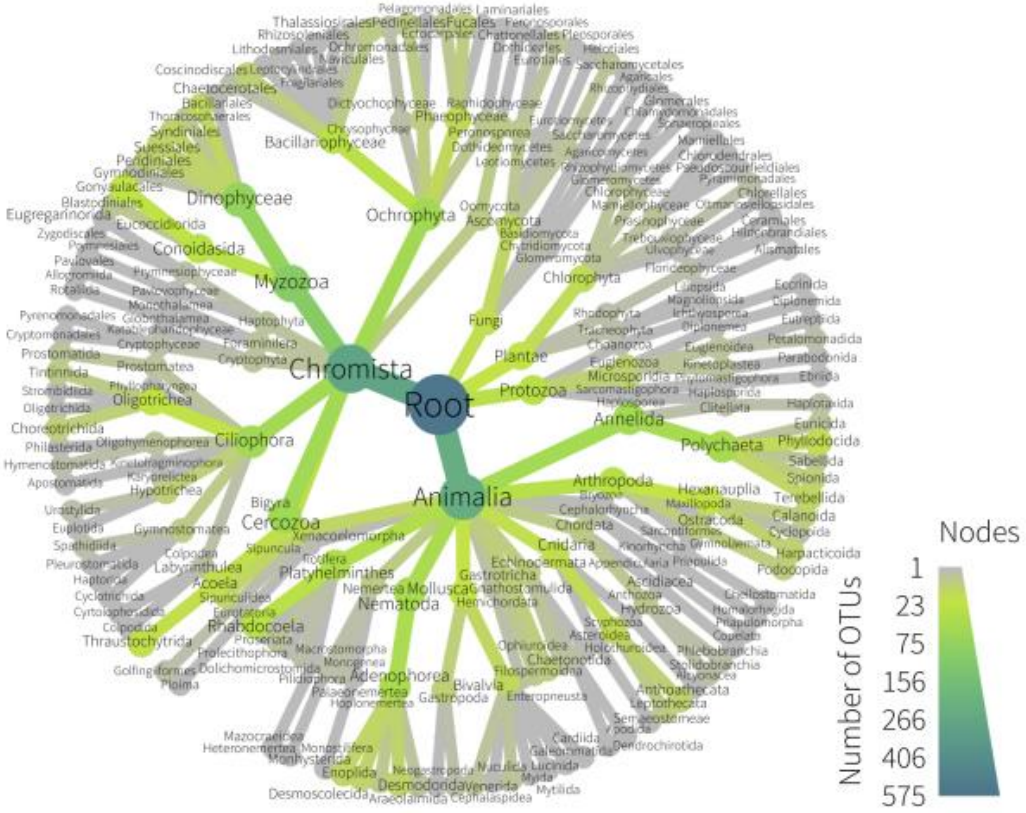
Taxonomic heat trees showing the number of OTUs across all samples for each habitat and assay. Each node (the circles) is a taxon and the edges (lines) show hierarchical relationships between taxa. The colour scale and the relative width of the node represent the number of taxa at each level.

5.1 Marine Lochs

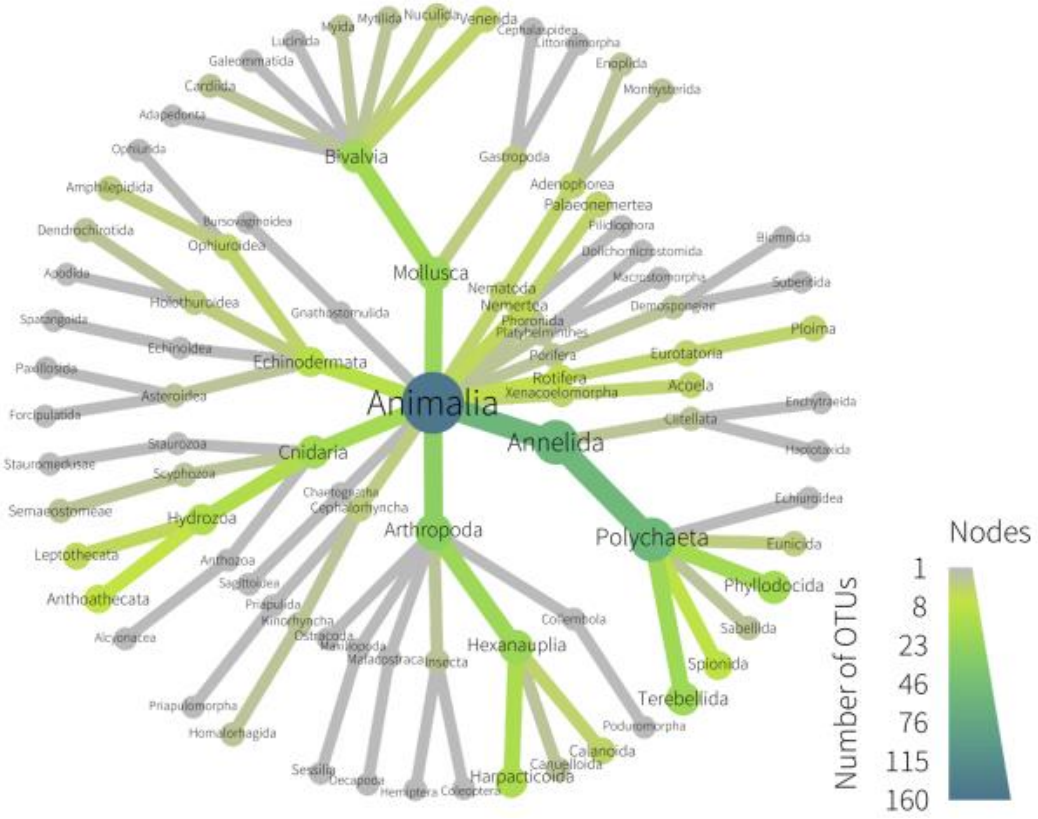
5.1.1 Bacteria



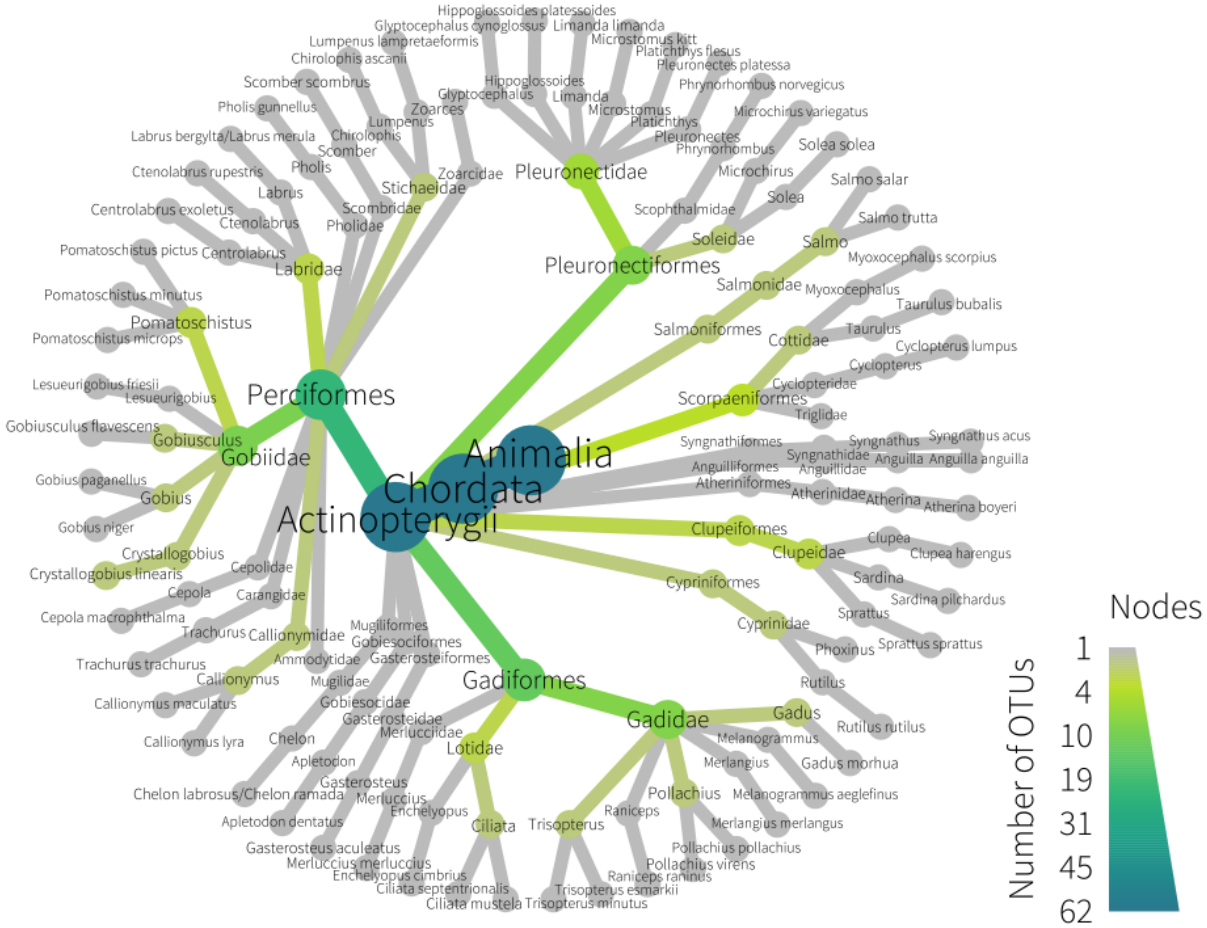
5.1.2 Sediment Eukaryotes



5.1.3 Sediment Invertebrates

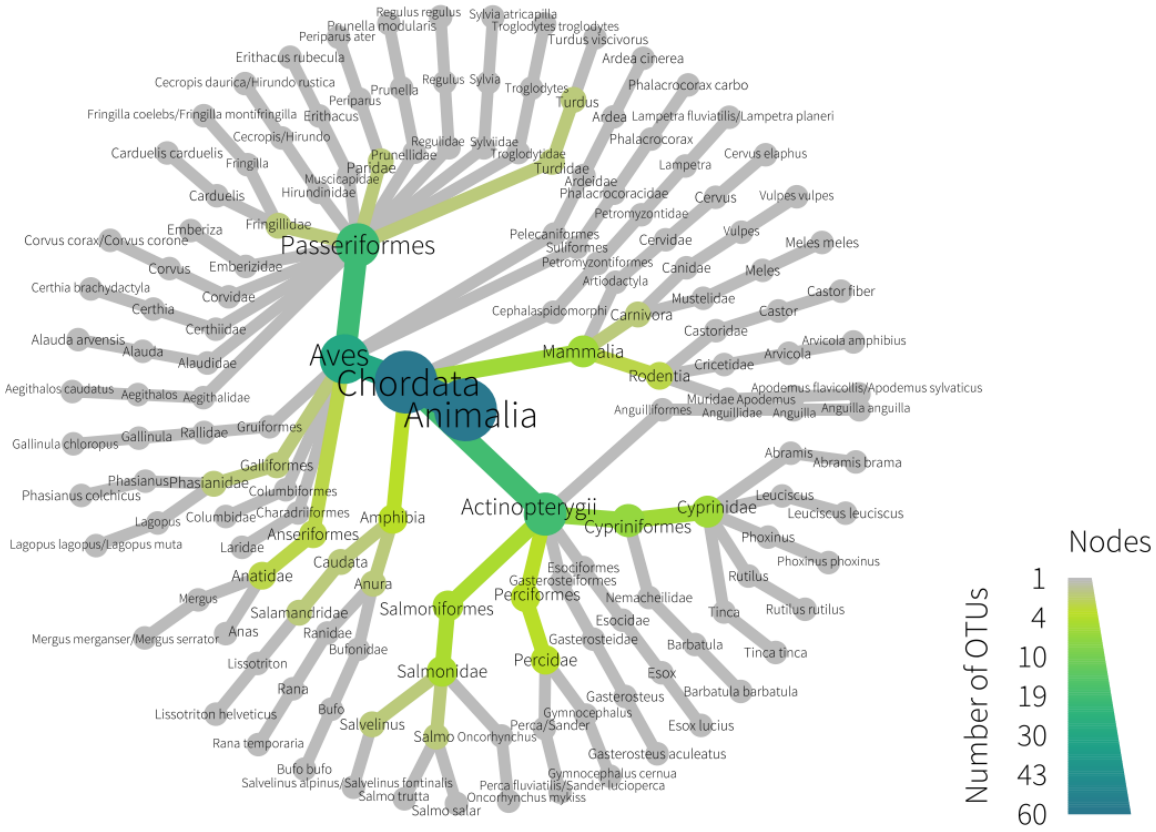


5.1.4 Fish

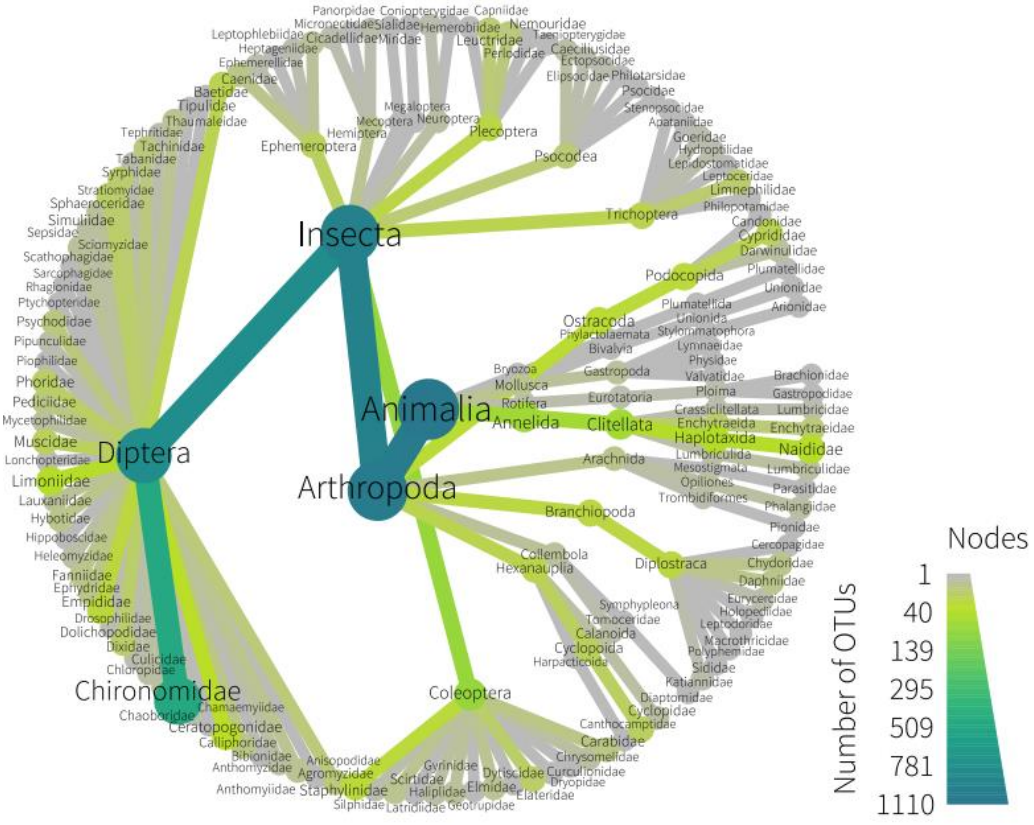

Animalia
Chordata
Actinopterygii
Perciformes
Gobiidae
Gobiusculus
Gobius
Pomatoschistus
Labridae
Stichaeidae
Zoarcidae
Scombridae
Pholidae
Pleuronectiformes
Pleuronectidae
Soleidae
Scophthalmidae
Salmoniformes
Salmonidae
Salmo
Cottidae
Scorpaeniformes
Cyclopteridae
Triglidae
Syngnathiformes
Syngnathidae
Syngnathus
Anguilliformes
Anguillidae
Anguilla
Atheriniformes
Atherinidae
Atherina
Clupeiformes
Clupeidae
Clupea
Cypriniformes
Cyprinidae
Sardina
Sprattus
Phoxinus
Rutilus
Gadiformes
Gadidae
Gadus
Melanogrammus
Merlangius
Pollachius
Raniceps
Trisopterus
Lotidae
Ciliata
Merluccidae
Gobiesociformes
Gobiesocidae
Gasterosteiformes
Gasterosteidae
Gasterosteus
Callionymidae
Callionymus
Mugiliformes
Mugilidae
Ammodytidae
Trachurus
Cepolidae
Cepola
Carangidae
Crystallogobius
Lesueurigobius
Pomatoschistus minutus
Pomatoschistus pictus
Pomatoschistus microps
Gobiusculus flavescens
Gobius paganellus
Gobius niger
Lesueurigobius friesii
Centrolabrus
Centrolabrus exoletus
Ctenolabrus
Ctenolabrus rupestris
Labrus
Labrus bergylta/Labrus merula
Pholis
Pholis gunnellus
Scomber
Scomber scombrus
Chirolophis
Chirolophis ascanii
Lumpenus
Lumpenus lampretaeformis
Zoarces
Glyptocephalus
Glyptocephalus cynoglossus
Hippoglossoides
Hippoglossoides platessoides
Limanda
Limanda limanda
Microstomus
Microstomus kitt
Platichthys
Platichthys flesus
Pleuronectes
Pleuronectes platessa
Phrynorhombus
Phrynorhombus norvegicus
Microchirus
Microchirus variegatus
Solea
Solea solea
Salmo salar
Salmo trutta
Myoxocephalus
Myoxocephalus scorpius
Taurulus
Taurulus bubalis
Cyclopterus
Cyclopterus lumpus
Syngnathus
Syngnathus acus
Anguilla
Anguilla anguilla
Atherina
Atherina boyeri
Clupea
Clupea harengus
Sardina
Sardina pilchardus
Sprattus
Sprattus sprattus
Rutilus
Rutilus rutilus
Gadus
Gadus morhua
Melanogrammus
Melanogrammus aeglefinus
Merlangius
Merlangius merlangus
Pollachius
Pollachius pollachius
Pollachius virens
Raniceps
Raniceps raninus
Trisopterus
Trisopterus esmarkii
Trisopterus minutus
Ciliata
Ciliata septentrionalis
Ciliata mustela
Enchelyopus
Enchelyopus cimbrius
Merluccius
Merluccius merluccius
Gasterosteus
Gasterosteus aculeatus
Apletodon
Apletodon dentatus
Chelon
Chelon labrosus/Chelon ramada
Callionymus
Callionymus lyra
Callionymus maculatus
Trachurus
Trachurus trachurus
Cepola
Cepola macrophthalma
Crystallogobius
Crystallogobius linearis
Gobius
Gobius niger
Gobiusculus
Gobiusculus flavescens
Lesueurigobius
Lesueurigobius friesii
Pomatoschistus
Pomatoschistus minutus
Pomatoschistus microps
Labridae
Centrolabrus
Centrolabrus exoletus
Ctenolabrus
Ctenolabrus rupestris
Labrus
Labrus bergylta/Labrus merula
Pholis
Pholis gunnellus
Scomber
Scomber scombrus
Chirolophis
Chirolophis ascanii
Lumpenus
Lumpenus lampretaeformis
Zoarcidae
Zoarces
Glyptocephalus
Glyptocephalus cynoglossus
Hippoglossoides
Hippoglossoides platessoides
Limanda
Limanda limanda
Microstomus
Microstomus kitt
Platichthys
Platichthys flesus
Pleuronectes
Pleuronectes platessa
Phrynorhombus
Phrynorhombus norvegicus
Microchirus
Microchirus variegatus
Solea
Solea solea
Salmo salar
Salmo trutta
Myoxocephalus
Myoxocephalus scorpius
Taurulus
Taurulus bubalis
Cyclopterus
Cyclopterus lumpus
Syngnathus
Syngnathus acus
Anguilla
Anguilla anguilla
Atherina
Atherina boyeri
Clupea
Clupea harengus
Sardina
Sardina pilchardus
Sprattus
Sprattus sprattus
Rutilus
Rutilus rutilus
Gadus
Gadus morhua
Melanogrammus
Melanogrammus aeglefinus
Merlangius
Merlangius merlangus
Pollachius
Pollachius pollachius
Pollachius virens
Raniceps
Raniceps raninus
Trisopterus
Trisopterus esmarkii
Trisopterus minutus
Ciliata
Ciliata septentrionalis
Ciliata mustela
Enchelyopus
Enchelyopus cimbrius
Merluccius
Merluccius merluccius
Gasterosteus
Gasterosteus aculeatus
Apletodon
Apletodon dentatus
Chelon
Chelon labrosus/Chelon ramada
Callionymus
Callionymus lyra
Callionymus maculatus
Trachurus
Trachurus trachurus
Cepola
Cepola macrophthalma
Crystallogobius
Crystallogobius linearis
Nodes
Number of OTUs
1
4
10
19
31
45
62

5.2 Freshwater Lochs

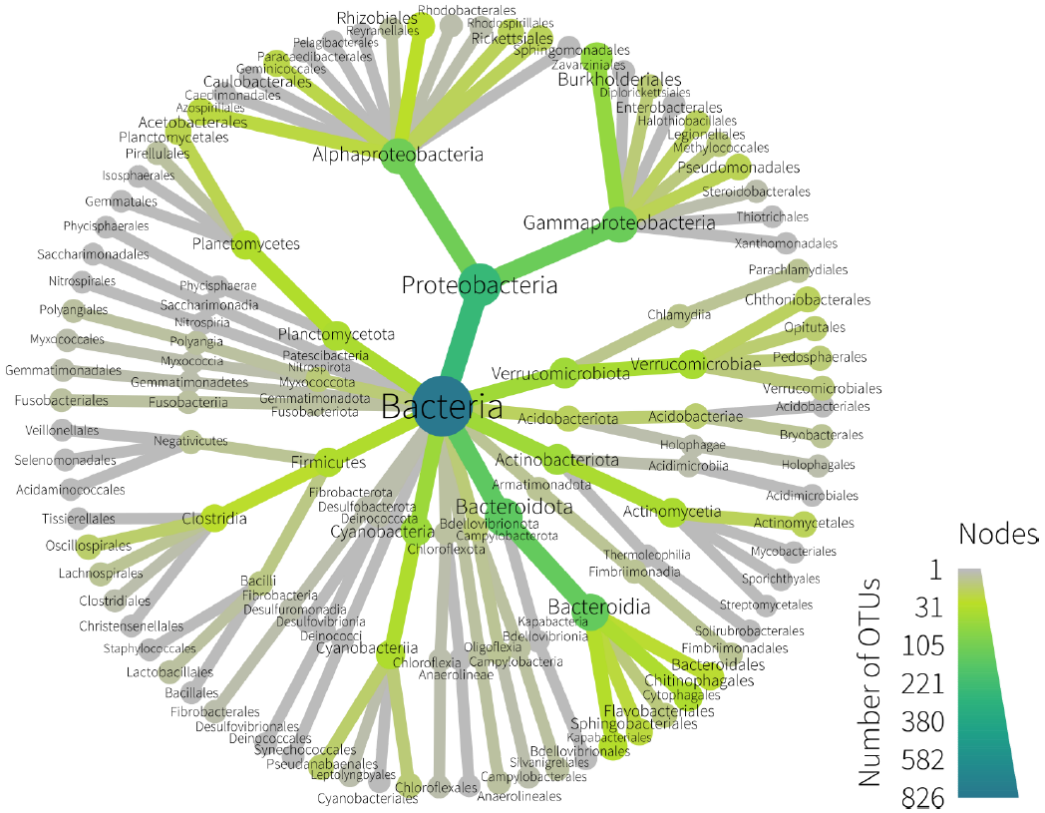
5.2.1 Vertebrates



5.2.2 Freshwater Insects

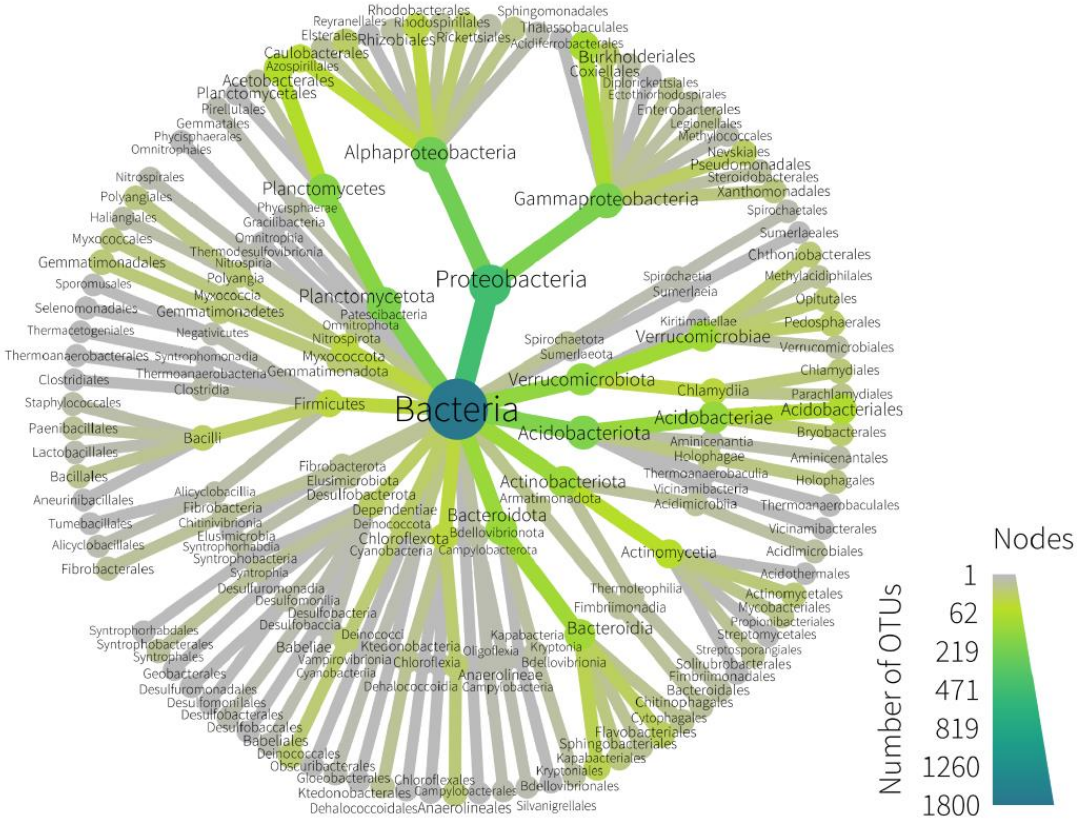


5.2.3 Bacteria

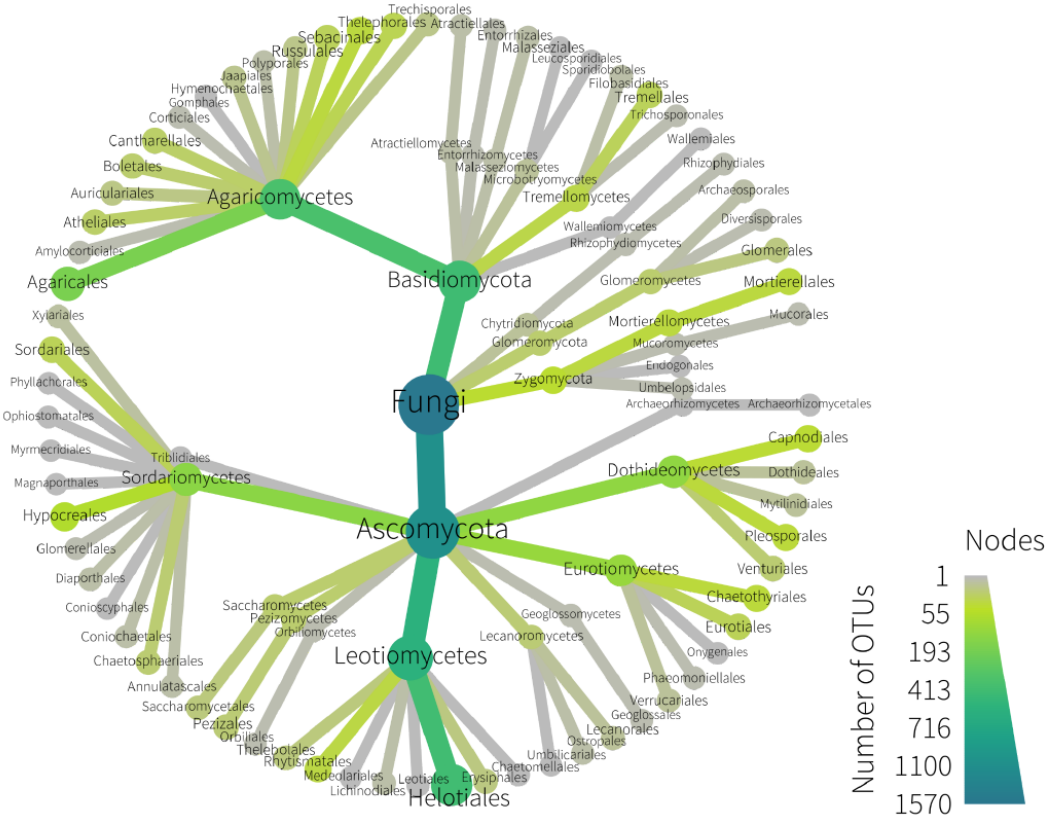


5.3 Woodland

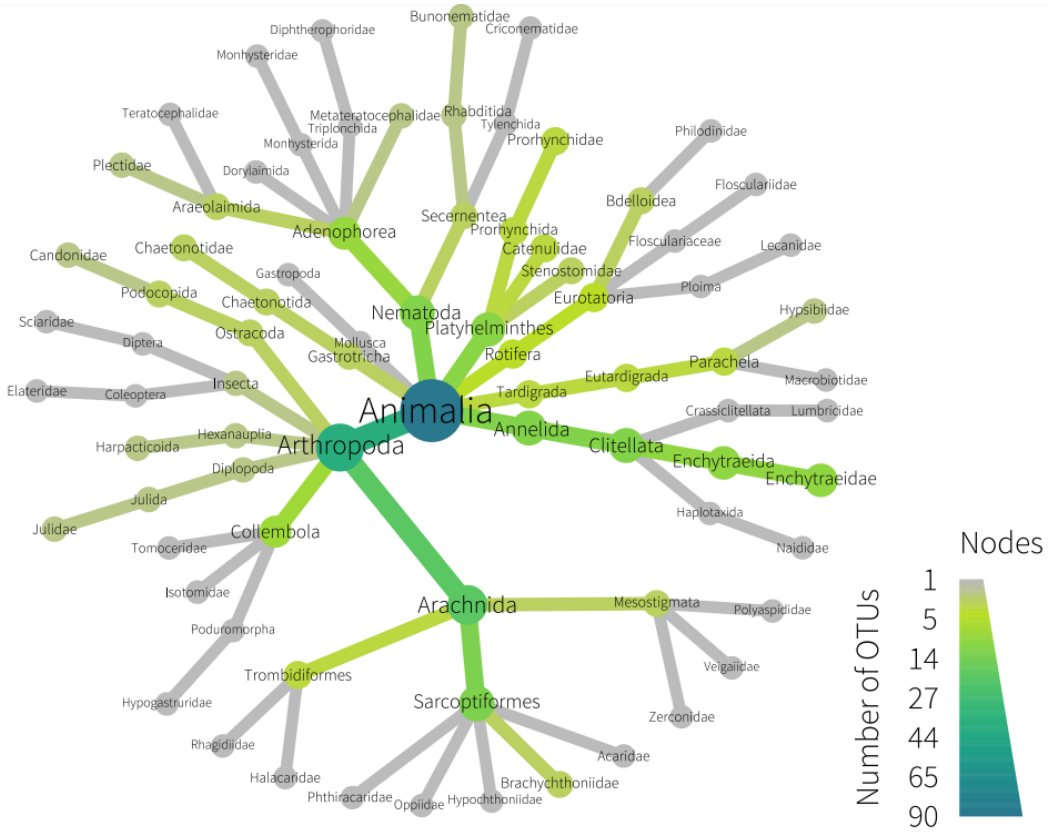
5.3.1 Bacteria



5.3.2 Fungi

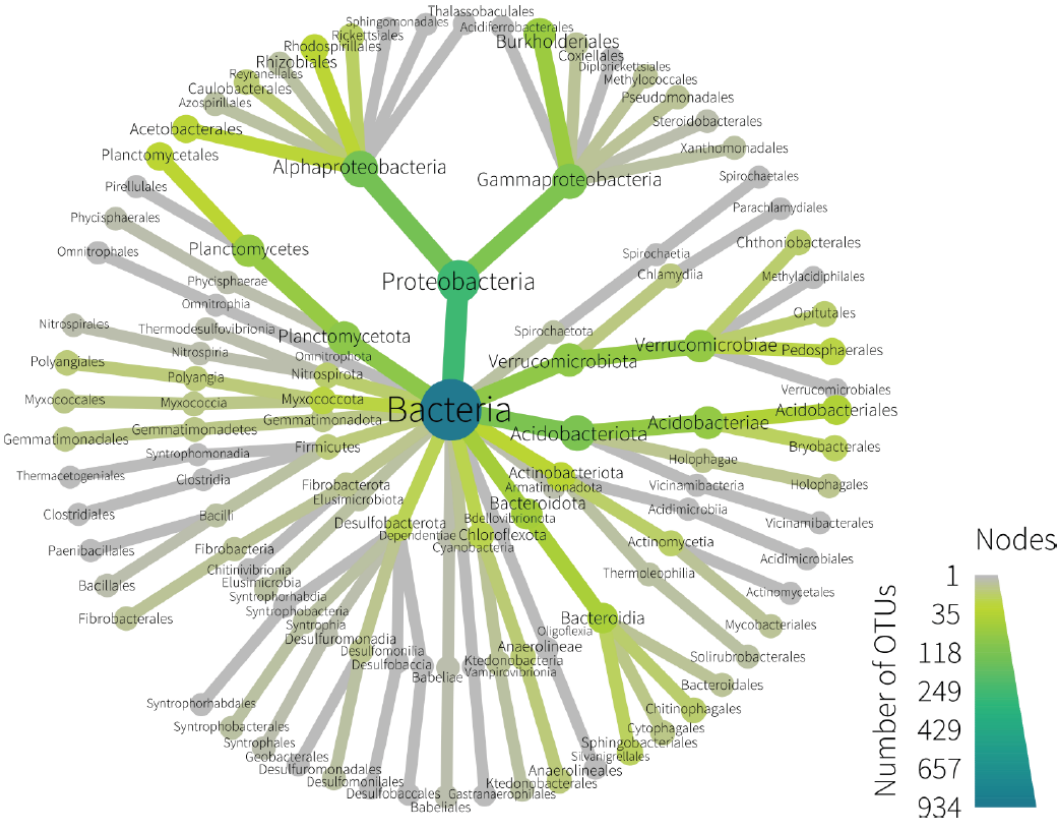


5.3.3 Soil Invertebrates

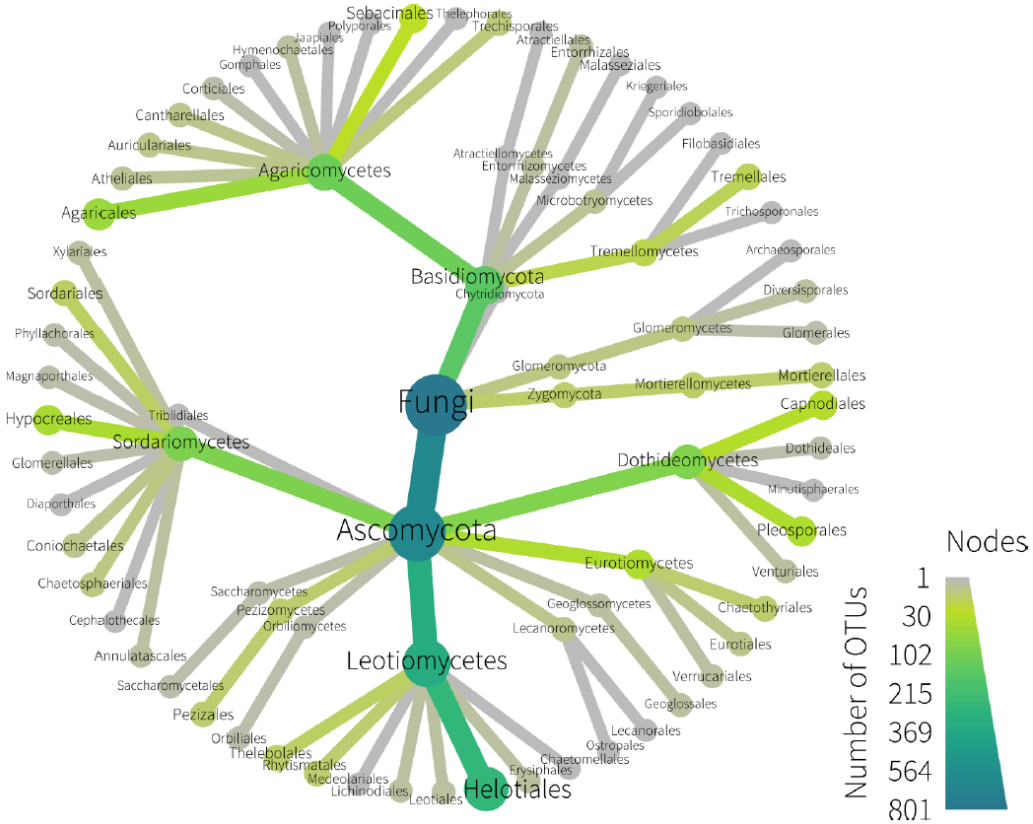


5.4 Peatland

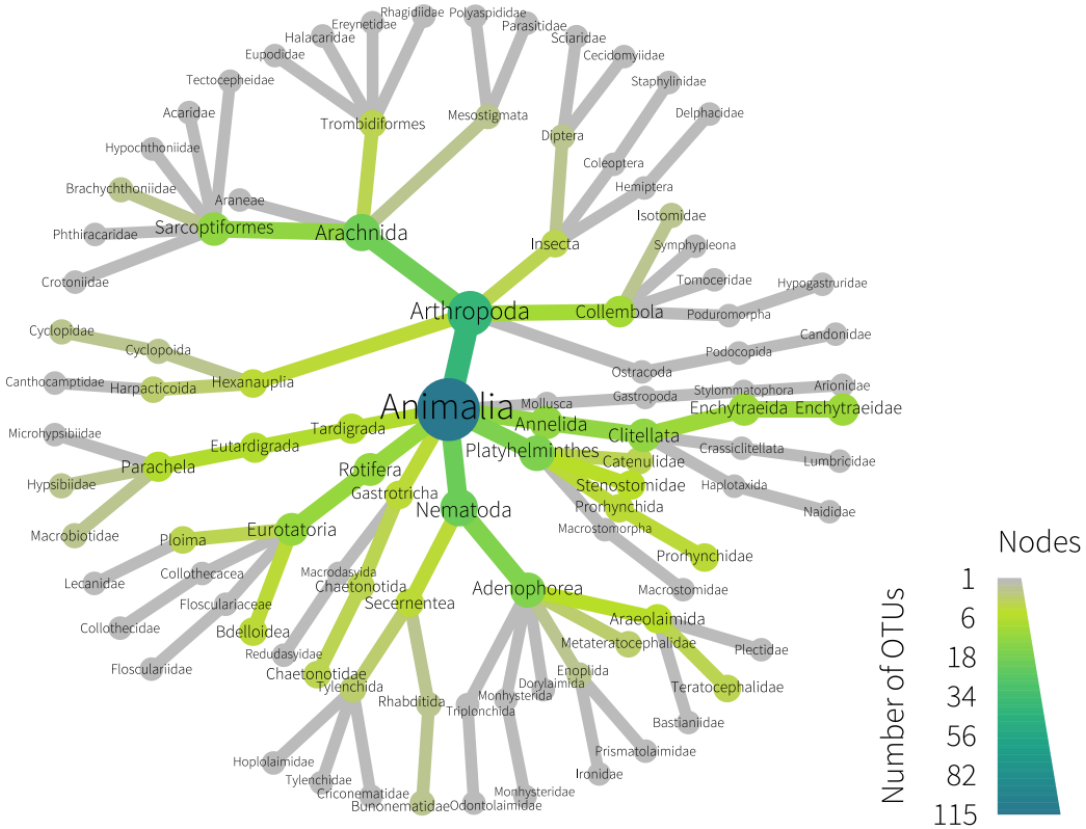
5.4.1 Bacteria



5.4.2 Fungi



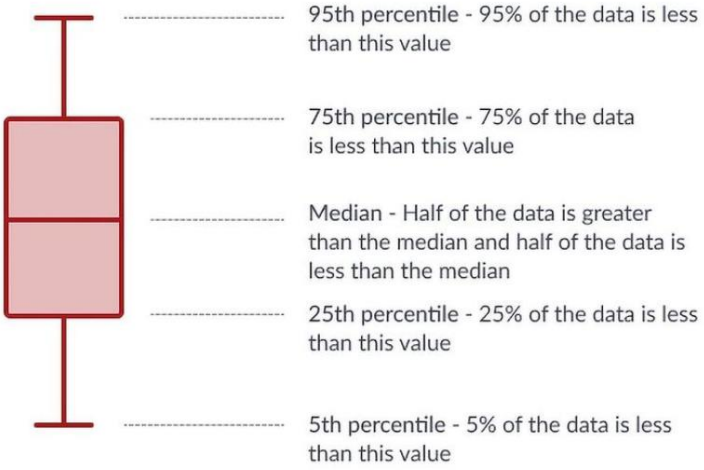
5.4.3 Soil Invertebrates



6 Sample Level Metrics

The figures in this section show the values and ranges for sample level metrics for all habitats and metrics interrogated in this project. The outputs of the models are shown below the plots. Results that showed clear and meaningful trends are discussed in the main report. In many cases there were statistically significant differences observed, but did not provide obvious and meaningful ecological inference – these are included in these Phase 2 Technical Appendices for completeness but not discussed further. For example, some marine biotopes had higher bacterial species richness than others – this is a useful descriptor of the biotopes but is not proposed as a core indictor for monitoring marine biotopes. For marine biotopes only Level-4 Particle Size Distribution biotopes are shown, as there were no community differences found for Level 5 biotopes.

Boxplots show the medians and percentiles for each group.



6.1 Marine Lochs

6.1.1 Bacteria

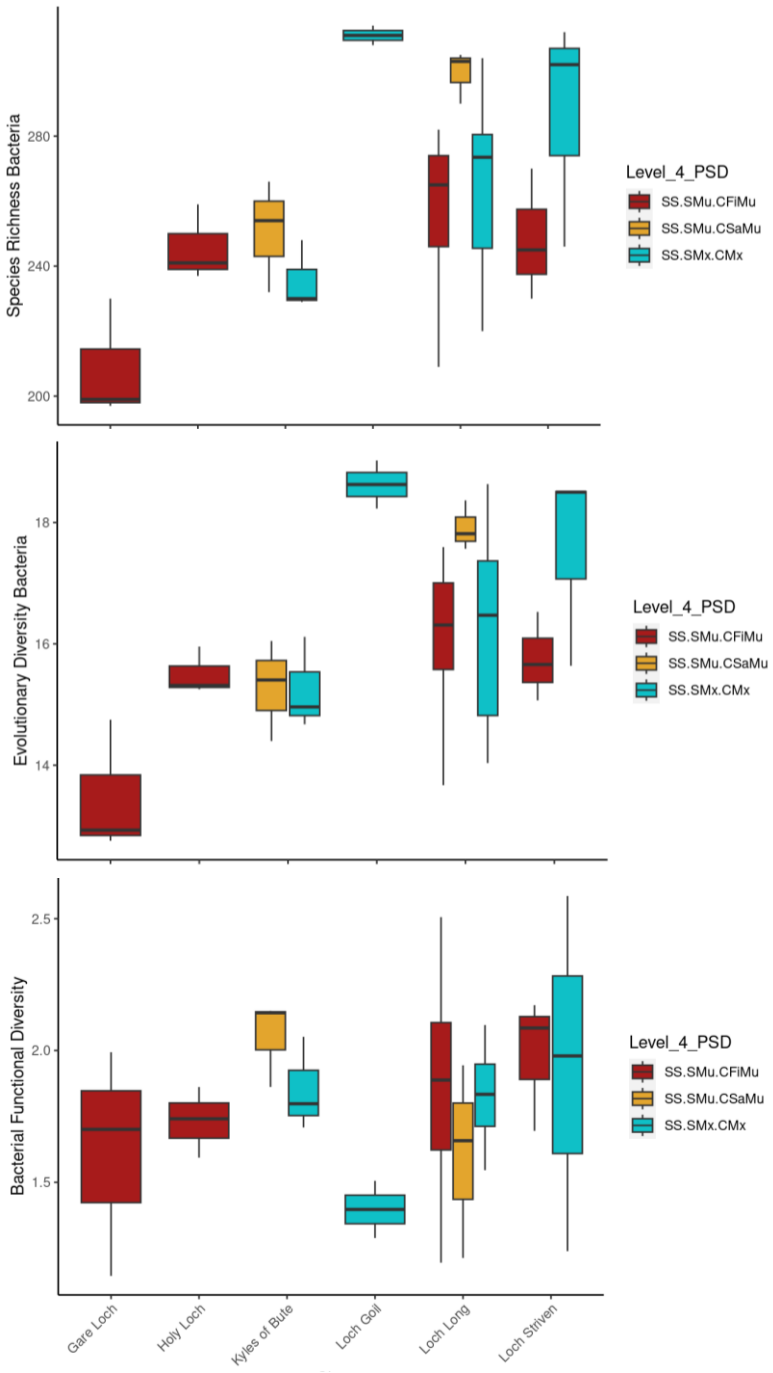


Figure 1: Medians and percentiles for Bacterial sample-level metrics.

```
## Species_Richness_Bacteria ~ Level_4_PSD + water_depth + salinity +
##     temperature + (1 | Site)
## $`emmeans of Level_4_PSD`
##  Level_4_PSD  emmean   SE   df lower.CL upper.CL
##  SS.SMu.CFiMu    260 15.1 4.96      221      298
##  SS.SMu.CSaMu    284 16.8 7.78      245      323
##  SS.SMx.CMx      281 15.8 5.53      242      320
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Level_4_PSD`
##  1                          estimate    SE   df t.ratio p.value
##  SS.SMu.CFiMu - SS.SMu.CSaMu   -24.16 10.41 48.6  -2.320  0.0624
##  SS.SMu.CFiMu - SS.SMx.CMx     -21.52  7.24 48.7  -2.973  0.0125
##  SS.SMu.CSaMu - SS.SMx.CMx       2.64 12.02 49.7   0.220  0.9738
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
##
## [3] "\"for each increase of 1 unit water_depth, Species_Richness_Bacteria increases 0.7719
units (p=0.3685)\""
## [4] "\"for each increase of 1 unit salinity, Species_Richness_Bacteria increases 52.3507 u
nits (p=0.0049)\""
## [5] "\"for each increase of 1 unit temperature, Species_Richness_Bacteria increases 13.820
1 units (p=0.0162)\""
## Overall model p value for Level_4_PSD=0.00292528112487104


## Evolutionary_Diversity_Bacteria ~ Level_4_PSD + water_depth +
##     salinity + temperature + (1 | Site)
## $`emmeans of Level_4_PSD`
##  Level_4_PSD  emmean    SE   df lower.CL upper.CL
##  SS.SMu.CFiMu   16.2 0.743 4.95     14.3     18.1
##  SS.SMu.CSaMu   16.9 0.850 8.58     15.0     18.9
##  SS.SMx.CMx     17.1 0.783 5.57     15.2     19.1
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Level_4_PSD`
##  1                          estimate    SE   df t.ratio p.value
##  SS.SMu.CFiMu - SS.SMu.CSaMu   -0.697 0.572 49.0  -1.217  0.4487
```

```
##  SS.SMu.CFiMu - SS.SMx.CMx     -0.892 0.397 49.1  -2.246  0.0734

##  SS.SMu.CSaMu - SS.SMx.CMx     -0.196 0.658 49.9  -0.298  0.9524

##

## Degrees-of-freedom method: kenward-roger

## P value adjustment: tukey method for comparing a family of 3 estimates

##

## [3] "\"for each increase of 1 unit water_depth, Evolutionary_Diversity_Bacteria increases
0.0527 units (p=0.2645)\""

## [4] "\"for each increase of 1 unit salinity, Evolutionary_Diversity_Bacteria increases 2.4
572 units (p=0.0127)\""

## [5] "\"for each increase of 1 unit temperature, Evolutionary_Diversity_Bacteria increases
0.8006 units (p=0.0111)\""

## Overall model p value for Level_4_PSD=0.0549411779515557


## Bacterial_Functional_Diversity ~ Level_4_PSD + water_depth +

##     salinity + temperature + (1 | Site)

## $`emmeans of Level_4_PSD`

##  Level_4_PSD  emmean    SE    df lower.CL upper.CL

##  SS.SMu.CFiMu   1.84 0.123  0.80    -1.07     4.76

##  SS.SMu.CSaMu   1.80 0.155 30.35     1.48     2.11

##  SS.SMx.CMx     1.82 0.118  4.16     1.50     2.14

##

## Degrees-of-freedom method: kenward-roger

## Confidence level used: 0.95

##

## $`pairwise differences of Level_4_PSD`

##  1                           estimate    SE   df t.ratio p.value

##  SS.SMu.CFiMu - SS.SMu.CSaMu   0.0469 0.182 31.3   0.257  0.9642

##  SS.SMu.CFiMu - SS.SMx.CMx     0.0218 0.119 49.4   0.183  0.9818

##  SS.SMu.CSaMu - SS.SMx.CMx    -0.0251 0.196 47.6  -0.128  0.9910

##

## Degrees-of-freedom method: kenward-roger

## P value adjustment: tukey method for comparing a family of 3 estimates

##

## [3] "\"for each increase of 1 unit water_depth, Bacterial_Functional_Diversity decreases 0
.0017 units (p=0.9083)\""

## [4] "\"for each increase of 1 unit salinity, Bacterial_Functional_Diversity increases 0.19
53 units (p=0.2802)\""

## [5] "\"for each increase of 1 unit temperature, Bacterial_Functional_Diversity decreases 0
.0316 units (p=0.6943)\""
```

6.1.2 Sediment Eukaryotes

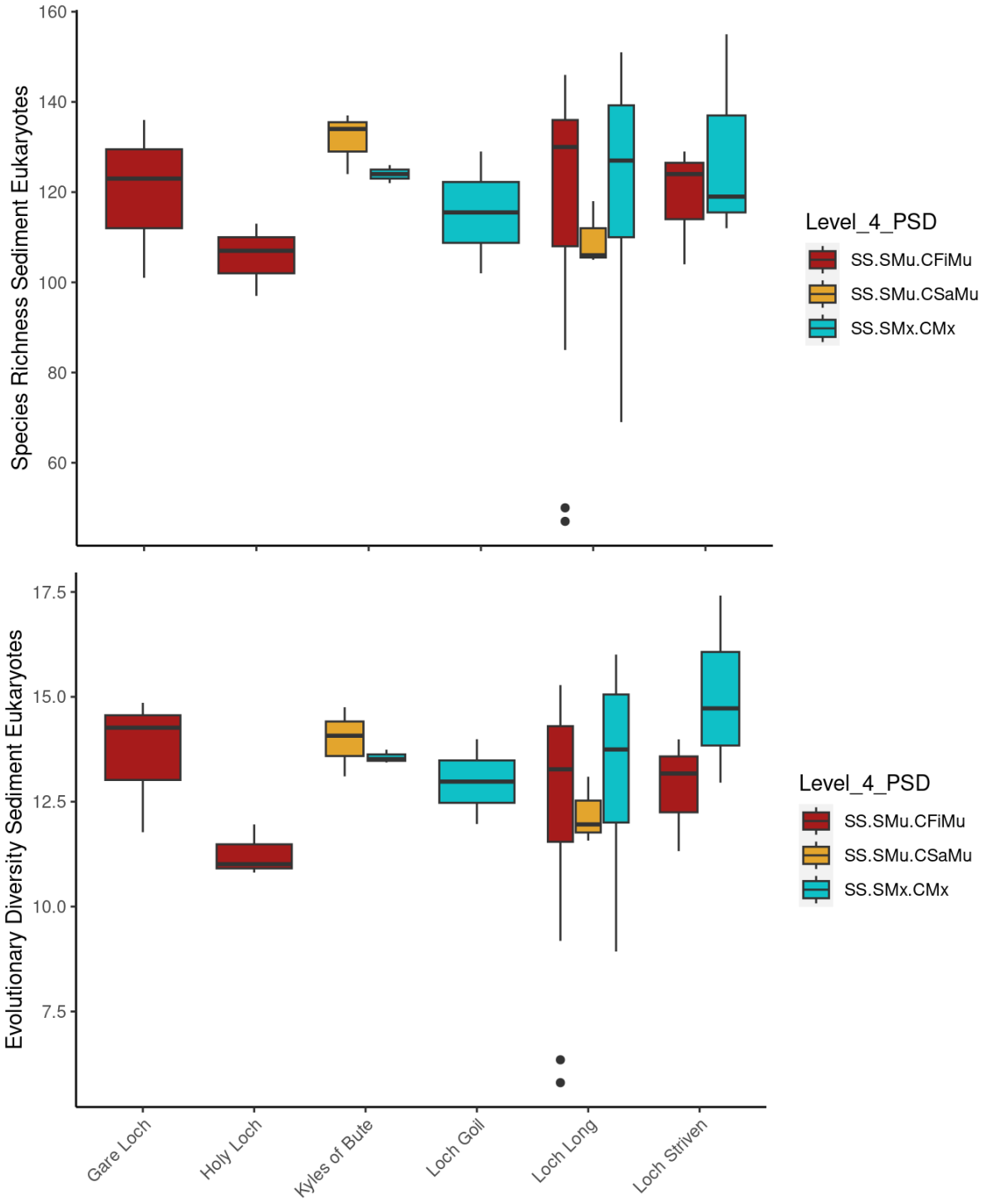


Figure 2: Medians and percentiles for Sediment Eukaryote sample-level metrics

```
## Species_Richness_Sediment_Eukaryotes ~ Level_4_PSD + water_depth +
##     salinity + temperature + (1 | Site)
## $`emmeans of Level_4_PSD`
##  Level_4_PSD  emmean    SE    df lower.CL upper.CL
##  SS.SMu.CFiMu    116  8.66  0.80    -87.9      321
##  SS.SMu.CSaMu    119 10.85 30.35     96.5      141
##  SS.SMx.CMx      123  8.28  4.16    100.4      146
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Level_4_PSD`
##  1                          estimate    SE   df t.ratio p.value
##  SS.SMu.CFiMu - SS.SMu.CSaMu    -2.18 12.80 31.3  -0.170  0.9841
##  SS.SMu.CFiMu - SS.SMx.CMx      -6.57  8.38 49.4  -0.784  0.7143
##  SS.SMu.CSaMu - SS.SMx.CMx      -4.39 13.72 47.6  -0.320  0.9451
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
##
## [3] "\"for each increase of 1 unit water_depth, Species_Richness_Sediment_Eukaryotes incre
ases 0.1055 units (p=0.9195)\""
## [4] "\"for each increase of 1 unit salinity, Species_Richness_Sediment_Eukaryotes increase
s 6.5168 units (p=0.6057)\""
## [5] "\"for each increase of 1 unit temperature, Species_Richness_Sediment_Eukaryotes incre
ases 2.3884 units (p=0.6721)\""
## Overall model p value for Level_4_PSD=0.715339598215641


## Evolutionary_Diversity_Sediment_Eukaryotes ~ Level_4_PSD + water_depth +
##     salinity + temperature + (1 | Site)
## $`emmeans of Level_4_PSD`
##  Level_4_PSD  emmean    SE    df lower.CL upper.CL
##  SS.SMu.CFiMu   12.5 0.769  1.52      8.0     17.1
##  SS.SMu.CSaMu   13.0 1.045 27.46     10.9     15.2
##  SS.SMx.CMx     13.7 0.765  5.13     11.7     15.6
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Level_4_PSD`
##  1                          estimate    SE   df t.ratio p.value
##  SS.SMu.CFiMu - SS.SMu.CSaMu   -0.506 1.189 35.4  -0.425  0.9055
```

```
##  SS.SMu.CFiMu - SS.SMx.CMx     -1.151 0.789 49.5  -1.459  0.3192

##  SS.SMu.CSaMu - SS.SMx.CMx     -0.645 1.286 48.1  -0.502  0.8709

##

## Degrees-of-freedom method: kenward-roger

## P value adjustment: tukey method for comparing a family of 3 estimates

##

## [3] "\"for each increase of 1 unit water_depth, Evolutionary_Diversity_Sediment_Eukaryotes
increases 0.0094 units (p=0.9233)\""

## [4] "\"for each increase of 1 unit salinity, Evolutionary_Diversity_Sediment_Eukaryotes in
creases 0.1911 units (p=0.8762)\""

## [5] "\"for each increase of 1 unit temperature, Evolutionary_Diversity_Sediment_Eukaryotes
increases 0.1121 units (p=0.8365)\""

## Overall model p value for Level_4_PSD=0.316930904968486
```

6.1.3 Sediment Invertebrates

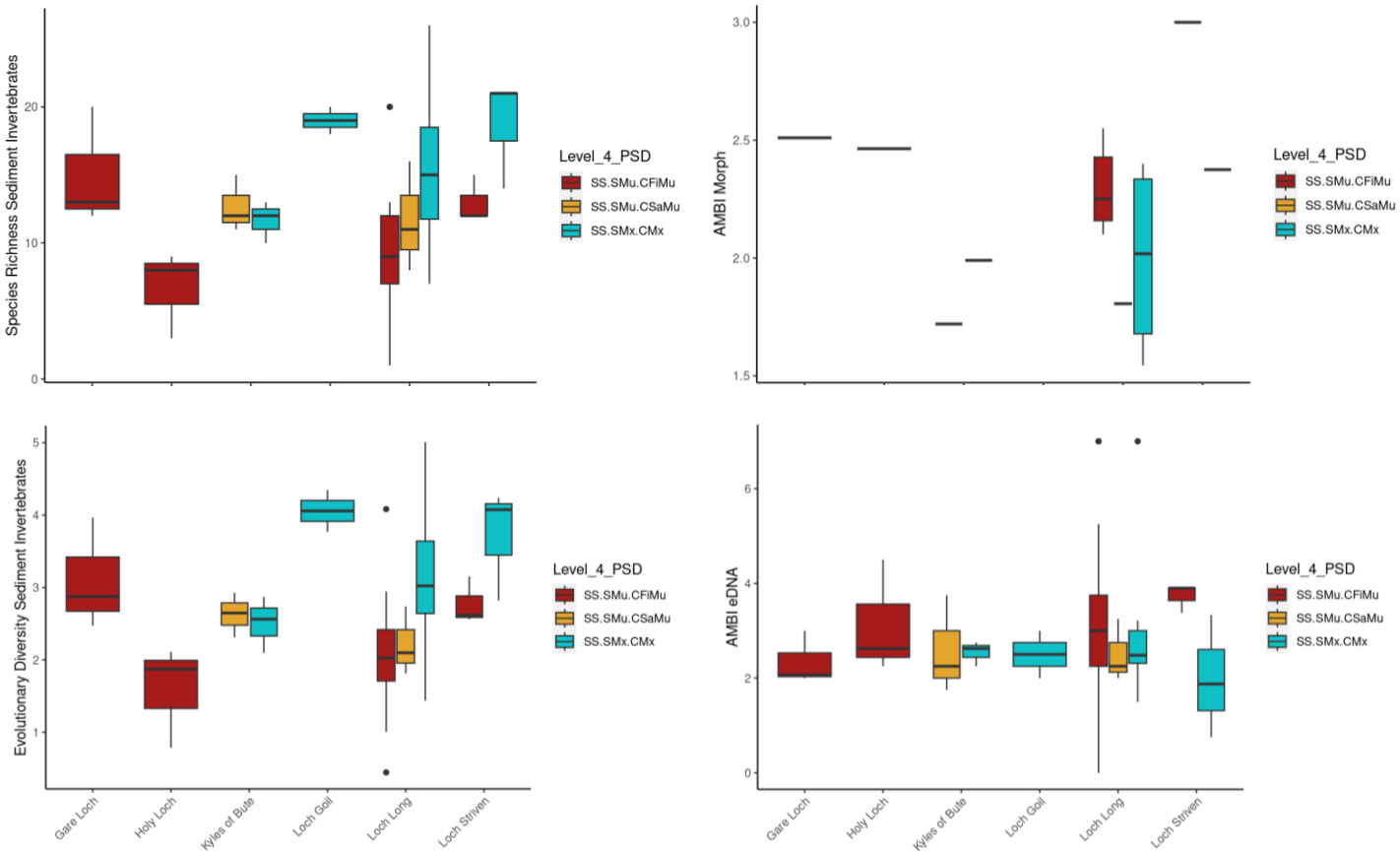


Figure 3: Medians and percentiles for Sediment Invertebrate sample-level metrics

```
## Species_Richness_Sediment_Invertebrates ~ Level_4_PSD + water_depth +
##     salinity + temperature + (1 | Site)
## $`emmeans of Level_4_PSD`
##  Level_4_PSD  emmean   SE    df lower.CL upper.CL
##  SS.SMu.CFiMu   10.4 1.58  4.46     6.17     14.6
##  SS.SMu.CSaMu   14.9 2.39 19.08     9.93     19.9
##  SS.SMx.CMx     15.0 1.73  6.16    10.78     19.2
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Level_4_PSD`
##  1                          estimate   SE   df t.ratio p.value
##  SS.SMu.CFiMu - SS.SMu.CSaMu  -4.5395 2.37 47.5  -1.915  0.1455
##  SS.SMu.CFiMu - SS.SMx.CMx    -4.5876 1.61 49.8  -2.844  0.0174
```

```
##  SS.SMu.CSaMu - SS.SMx.CMx    -0.0481 2.61 49.4  -0.018  0.9998

##

## Degrees-of-freedom method: kenward-roger

## P value adjustment: tukey method for comparing a family of 3 estimates

##

## [3] "\"for each increase of 1 unit water_depth, Species_Richness_Sediment_Invertebrates de
creases 0.3946 units (p=0.0485)\""

## [4] "\"for each increase of 1 unit salinity, Species_Richness_Sediment_Invertebrates incre
ases 1.9163 units (p=0.5182)\""

## [5] "\"for each increase of 1 unit temperature, Species_Richness_Sediment_Invertebrates de
creases 2.2423 units (p=0.0641)\""

## Overall model p value for Level_4_PSD=0.00671031298746801


## Evolutionary_Diversity_Sediment_Invertebrates ~ Level_4_PSD +

##     water_depth + salinity + temperature + (1 | Site)

## $`emmeans of Level_4_PSD`

##  Level_4_PSD  emmean    SE    df lower.CL upper.CL

##  SS.SMu.CFiMu   2.25 0.285  0.95    -1.91     6.40

##  SS.SMu.CSaMu   2.90 0.362 29.16     2.16     3.64

##  SS.SMx.CMx     2.99 0.276  3.84     2.22     3.77

##

## Degrees-of-freedom method: kenward-roger

## Confidence level used: 0.95

##

## $`pairwise differences of Level_4_PSD`

##  1                          estimate    SE   df t.ratio p.value

##  SS.SMu.CFiMu - SS.SMu.CSaMu  -0.6475 0.426 31.4  -1.520  0.2956

##  SS.SMu.CFiMu - SS.SMx.CMx    -0.7457 0.279 47.6  -2.673  0.0272

##  SS.SMu.CSaMu - SS.SMx.CMx    -0.0983 0.458 45.8  -0.214  0.9750

##

## Degrees-of-freedom method: kenward-roger

## P value adjustment: tukey method for comparing a family of 3 estimates

##

## [1] "\"for each increase of 1 unit Level_4_PSDSS.SMu.CSaMu, Evolutionary_Diversity_Sedimen
t_Invertebrates increases 0.6475 units (p=0.0851)\""

## [2] "\"for each increase of 1 unit Level_4_PSDSS.SMx.CMx, Evolutionary_Diversity_Sediment_
Invertebrates increases 0.7457 units (p=0.0082)\""

## [3] "\"for each increase of 1 unit water_depth, Evolutionary_Diversity_Sediment_Invertebra
tes decreases 0.086 units (p=0.017)\""

## [4] "\"for each increase of 1 unit salinity, Evolutionary_Diversity_Sediment_Invertebrates
decreases 0.0367 units (p=0.9305)\""

## [5] "\"for each increase of 1 unit temperature, Evolutionary_Diversity_Sediment_Invertebra
tes decreases 0.6641 units (p=0.001)\""

## Overall model p value for Level_4_PSD=0.0120269461819754
```

6.1.4 Fish

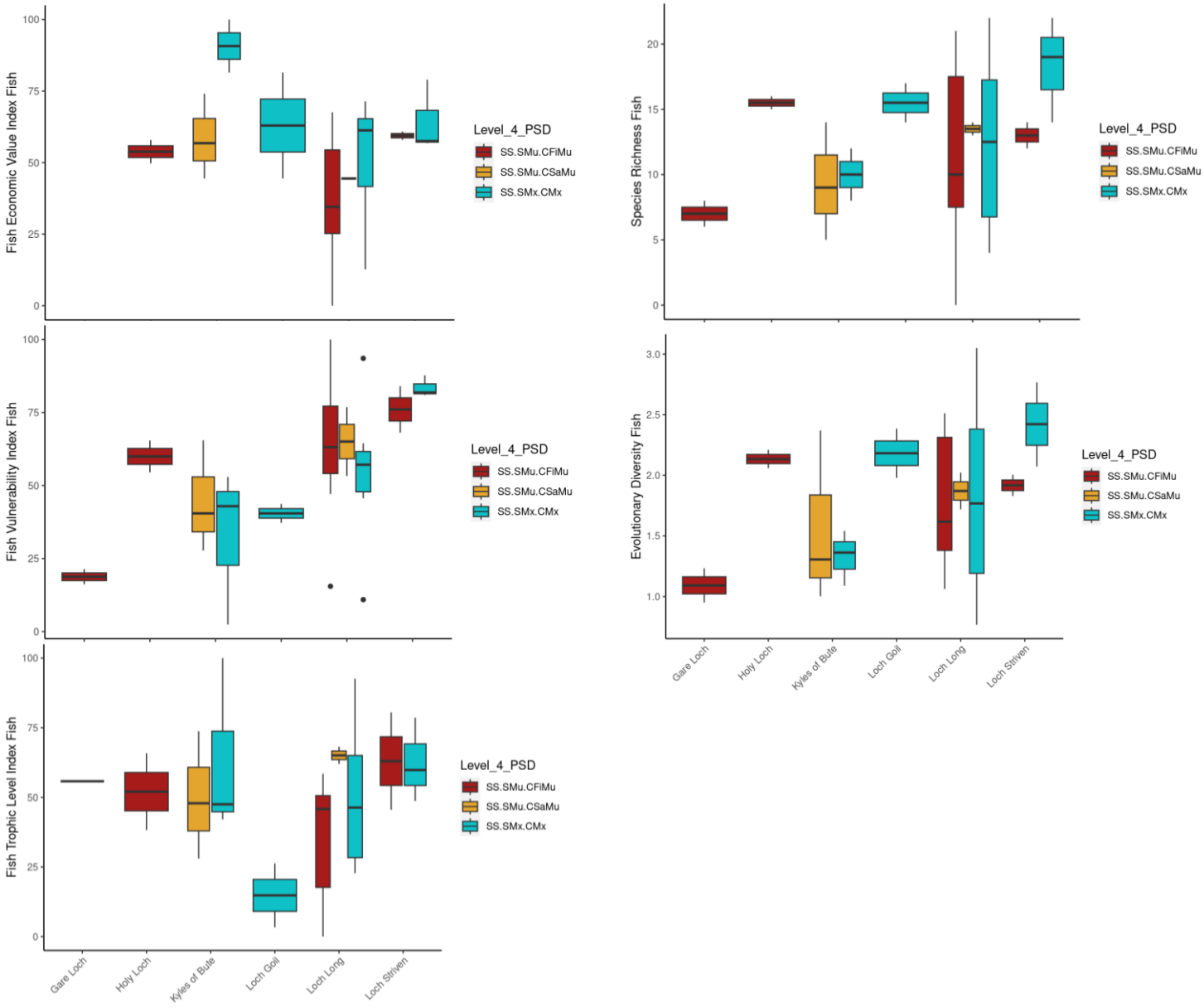


Figure 4: Medians and percentiles for Fish sample-level metrics

```
## Species_Richness_Fish ~ Level_4_PSD + water_depth + salinity +
##     temperature + (1 | Site)
## $`emmeans of Level_4_PSD`
##  Level_4_PSD  emmean   SE    df lower.CL upper.CL
##  SS.SMu.CFiMu   13.3 2.05  4.85     7.96     18.6
##  SS.SMu.CSaMu   14.0 3.09 16.18     7.44     20.5
##  SS.SMx.CMx     13.1 2.16  5.63     7.69     18.5
##
```

```
## Degrees-of-freedom method: kenward-roger

## Confidence level used: 0.95

##

## $`pairwise differences of Level_4_PSD`

##  1                           estimate   SE   df t.ratio p.value

##  SS.SMu.CFiMu - SS.SMu.CSaMu   -0.713 3.14 33.8  -0.227  0.9719

##  SS.SMu.CFiMu - SS.SMx.CMx      0.202 2.12 36.0   0.095  0.9950

##  SS.SMu.CSaMu - SS.SMx.CMx      0.915 3.38 35.3   0.271  0.9605

##

## Degrees-of-freedom method: kenward-roger

## P value adjustment: tukey method for comparing a family of 3 estimates

##

## [3] "\"for each increase of 1 unit water_depth, Species_Richness_Fish decreases 0.5635 uni
ts (p=0.0422)\""

## [4] "\"for each increase of 1 unit salinity, Species_Richness_Fish increases 9.5619 units
(p=0.0226)\""

## [5] "\"for each increase of 1 unit temperature, Species_Richness_Fish decreases 0.4364 uni
ts (p=0.7804)\""

## Overall model p value for Level_4_PSD=0.959989235946926


## Evolutionary_Diversity_Fish ~ Level_4_PSD + water_depth + salinity +

##     temperature + (1 | Site)

## $`emmeans of Level_4_PSD`

##  Level_4_PSD  emmean    SE    df lower.CL upper.CL

##  SS.SMu.CFiMu   1.94 0.213  5.06     1.39     2.48

##  SS.SMu.CSaMu   2.05 0.318 15.89     1.38     2.72

##  SS.SMx.CMx     1.79 0.225  5.58     1.23     2.36

##

## Degrees-of-freedom method: kenward-roger

## Confidence level used: 0.95

##

## $`pairwise differences of Level_4_PSD`

##  1                           estimate    SE   df t.ratio p.value

##  SS.SMu.CFiMu - SS.SMu.CSaMu   -0.113 0.320 33.5  -0.354  0.9333

##  SS.SMu.CFiMu - SS.SMx.CMx      0.143 0.218 35.0   0.658  0.7892

##  SS.SMu.CSaMu - SS.SMx.CMx      0.256 0.344 34.5   0.745  0.7387

##

## Degrees-of-freedom method: kenward-roger

## P value adjustment: tukey method for comparing a family of 3 estimates

##

## [3] "\"for each increase of 1 unit water_depth, Evolutionary_Diversity_Fish decreases 0.07
66 units (p=0.0081)\""

## [4] "\"for each increase of 1 unit salinity, Evolutionary_Diversity_Fish increases 1.2216
units (p=0.0069)\""
```

```
## [5] "\"for each increase of 1 unit temperature, Evolutionary_Diversity_Fish decreases 0.13
97 units (p=0.3844)\""

## Overall model p value for Level_4_PSD=0.694842634584944


## Fish_Economic_Value_Index_Fish ~ Level_4_PSD + water_depth +
##     salinity + temperature + (1 | Site)

## $`emmeans of Level_4_PSD`

##  Level_4_PSD  emmean    SE   df lower.CL upper.CL
##  SS.SMu.CFiMu   58.7  9.65 4.51     33.1     84.4
##  SS.SMu.CSaMu   64.7 12.84 9.40     35.9     93.6
##  SS.SMx.CMx     58.2 10.15 5.38     32.7     83.8
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Level_4_PSD`
##  1                           estimate    SE   df t.ratio p.value
##  SS.SMu.CFiMu - SS.SMu.CSaMu   -6.011 10.93 30.0  -0.550  0.8472
##  SS.SMu.CFiMu - SS.SMx.CMx      0.504  8.65 29.6   0.058  0.9981
##  SS.SMu.CSaMu - SS.SMx.CMx      6.515 13.58 29.8   0.480  0.8813
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates


## [3] "\"for each increase of 1 unit water_depth, Fish_Economic_Value_Index_Fish decreases 0
.9634 units (p=0.3024)\""

## [4] "\"for each increase of 1 unit salinity, Fish_Economic_Value_Index_Fish decreases 3.04
25 units (p=0.8781)\""

## [5] "\"for each increase of 1 unit temperature, Fish_Economic_Value_Index_Fish increases 1
0.4516 units (p=0.0716)\""

## Overall model p value for Level_4_PSD=0.836958890347074


## Fish_Vulnerability_Index_Fish ~ Level_4_PSD + water_depth + salinity +
##     temperature + (1 | Site)

## $`emmeans of Level_4_PSD`

##  Level_4_PSD  emmean   SE    df lower.CL upper.CL
##  SS.SMu.CFiMu   59.8 8.36  1.01    -43.3    162.9
##  SS.SMu.CSaMu   49.7 9.93 20.64     29.0     70.4
##  SS.SMx.CMx     56.5 6.75  6.93     40.5     72.5
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Level_4_PSD`
```

```
##  1                           estimate    SE   df t.ratio p.value
##  SS.SMu.CFiMu - SS.SMu.CSaMu    10.09 12.52 17.6   0.806  0.7046
##  SS.SMu.CFiMu - SS.SMx.CMx       3.32  8.83 30.7   0.376  0.9254
##  SS.SMu.CSaMu - SS.SMx.CMx      -6.77 12.64 33.0  -0.536  0.8544
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
##
## [3] "\"for each increase of 1 unit water_depth, Fish_Vulnerability_Index_Fish decreases 0.
5841 units (p=0.5808)\""
## [4] "\"for each increase of 1 unit salinity, Fish_Vulnerability_Index_Fish increases 25.96
19 units (p=0.0435)\""
## [5] "\"for each increase of 1 unit temperature, Fish_Vulnerability_Index_Fish decreases 11
.3503 units (p=0.0452)\""
## Overall model p value for Level_4_PSD=0.581091054756048

## Fish_Trophic_Level_Index_Fish ~ Level_4_PSD + water_depth + salinity +
##     temperature + (1 | Site)
## $`emmeans of Level_4_PSD`
##  Level_4_PSD  emmean    SE    df lower.CL upper.CL
##  SS.SMu.CFiMu   40.6  9.68  4.48     14.8     66.3
##  SS.SMu.CSaMu   51.7 14.32 15.18     21.3     82.2
##  SS.SMx.CMx     55.3  9.82  5.79     31.0     79.5
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Level_4_PSD`
##  1                           estimate   SE   df t.ratio p.value
##  SS.SMu.CFiMu - SS.SMu.CSaMu   -11.18 14.9 29.3  -0.749  0.7366
##  SS.SMu.CFiMu - SS.SMx.CMx     -14.71 10.7 32.9  -1.370  0.3679
##  SS.SMu.CSaMu - SS.SMx.CMx      -3.53 16.3 32.1  -0.216  0.9746
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
##
## [3] "\"for each increase of 1 unit water_depth, Fish_Trophic_Level_Index_Fish increases 1.
1908 units (p=0.3608)\""
## [4] "\"for each increase of 1 unit salinity, Fish_Trophic_Level_Index_Fish decreases 8.823
3 units (p=0.643)\""
## [5] "\"for each increase of 1 unit temperature, Fish_Trophic_Level_Index_Fish increases 2.
6043 units (p=0.7206)\""
## Overall model p value for Level_4_PSD=0.309937992242174
```

6.2 Freshwater Lochs

6.2.1 Bacteria

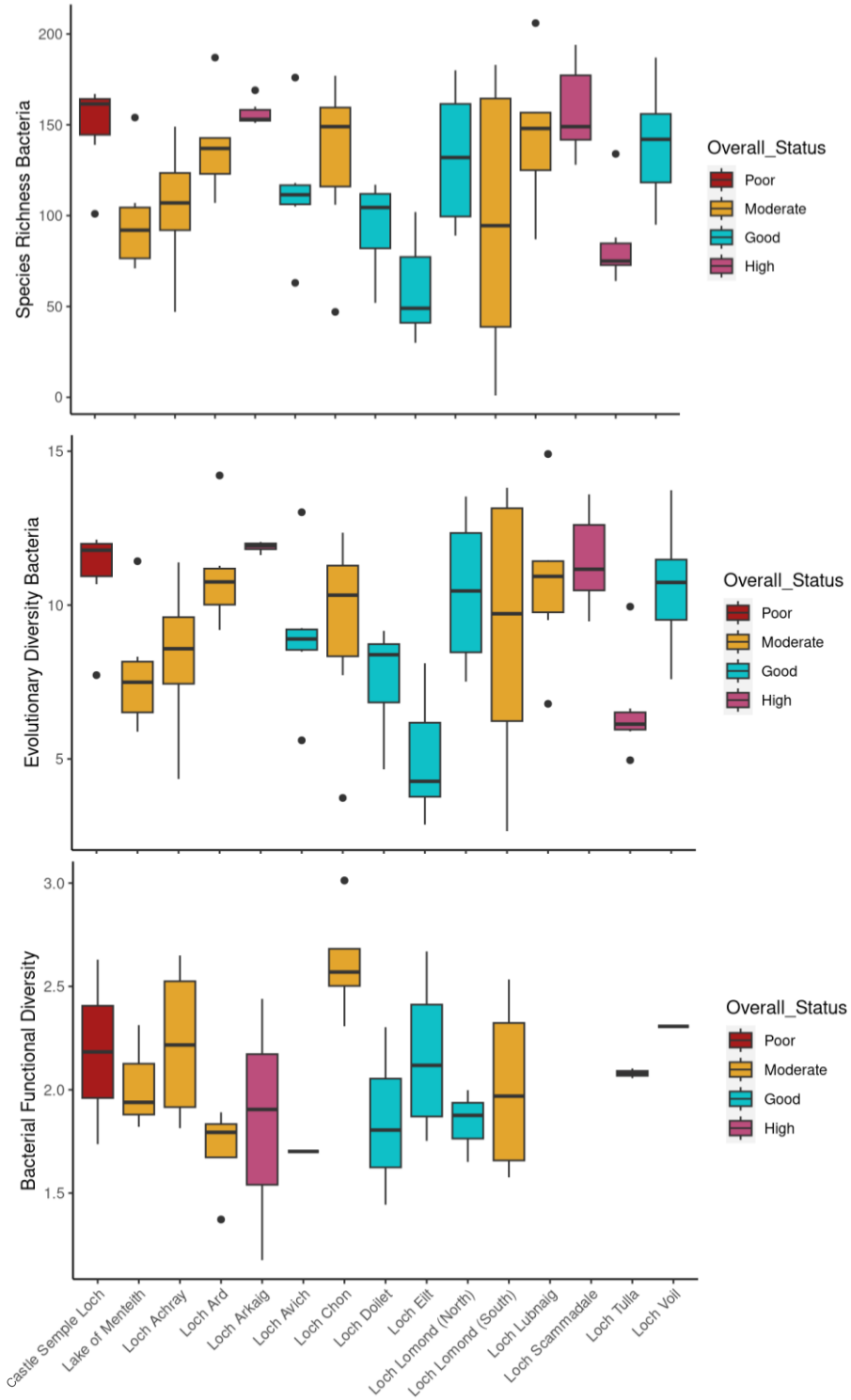


Figure 5: Medians and percentiles for Bacteria sample-level metrics. Contains SEPA data © Scottish Environment Protection Agency and database right (2023). All rights reserved.

```
## Species_Richness_Bacteria ~ Overall_Status + pH_field + Conductivity_field +
##     (1 | Site)
## $`emmeans of Overall_Status`
##  Overall_Status emmean   SE   df lower.CL upper.CL
##  Poor              149 28.1 10.1     86.6      211
##  Moderate          120 11.7 10.8     93.8      146
##  Good              107 12.6 10.3     78.7      135
##  High              134 16.5 10.6     97.0      170
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Overall_Status`
##  1               estimate   SE   df t.ratio p.value
##  Poor - Moderate     29.4 30.3 10.1   0.970  0.7687
##  Poor - Good         42.2 30.8 10.2   1.370  0.5432
##  Poor - High         15.5 32.7 10.4   0.473  0.9635
##  Moderate - Good     12.9 17.5 10.9   0.738  0.8796
##  Moderate - High    -13.9 20.7 11.2  -0.671  0.9057
##  Good - High        -26.8 20.6 10.2  -1.302  0.5815
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 4 estimates
##
## [4] "\"for each increase of 1 unit pH_field, Species_Richness_Bacteria increases 2.1443 un
its (p=0.8395)\""
## [5] "\"for each increase of 1 unit Conductivity_field, Species_Richness_Bacteria increases
52.2641 units (p=0.0752)\""
## Overall model p value for Overall_Status=0.444601670050066


## Evolutionary_Diversity_Bacteria ~ Overall_Status + pH_field +
##     Conductivity_field + (1 | Site)
## $`emmeans of Overall_Status`
##  Overall_Status emmean    SE   df lower.CL upper.CL
##  Poor            11.01 1.941 10.1     6.69     15.3
##  Moderate         9.41 0.813 10.9     7.62     11.2
##  Good             8.48 0.872 10.3     6.55     10.4
##  High            10.04 1.140 10.6     7.52     12.6
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Overall_Status`
```

```
##  1               estimate   SE   df t.ratio p.value
##  Poor - Moderate    1.597 2.09 10.1   0.763  0.8693
##  Poor - Good        2.521 2.13 10.2   1.182  0.6507
##  Poor - High        0.963 2.26 10.4   0.426  0.9728
##  Moderate - Good    0.923 1.21 10.9   0.766  0.8681
##  Moderate - High   -0.634 1.43 11.2  -0.445  0.9693
##  Good - High       -1.558 1.42 10.2  -1.095  0.6999
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 4 estimates
##
## [4] "\"for each increase of 1 unit pH_field, Evolutionary_Diversity_Bacteria increases 0.1
768 units (p=0.799)\""
## [5] "\"for each increase of 1 unit Conductivity_field, Evolutionary_Diversity_Bacteria inc
reases 2.9131 units (p=0.1236)\""
## Overall model p value for Overall_Status=0.569952242183404


## Bacterial_Functional_Diversity ~ Overall_Status + pH_field +
##     Conductivity_field + (1 | Site)
## $`emmeans of Overall_Status`
##  Overall_Status emmean    SE    df lower.CL upper.CL
##  Poor             2.23 0.325 12.91     1.52     2.93
##  Moderate         2.17 0.126  6.13     1.86     2.47
##  Good             1.93 0.145  9.48     1.60     2.25
##  High             1.88 0.232 10.28     1.36     2.39
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Overall_Status`
##  1               estimate    SE    df t.ratio p.value
##  Poor - Moderate   0.0607 0.343 11.58   0.177  0.9979
##  Poor - Good       0.2971 0.358 12.60   0.830  0.8394
##  Poor - High       0.3503 0.407 12.09   0.861  0.8244
##  Moderate - Good   0.2364 0.198  8.68   1.196  0.6445
##  Moderate - High   0.2896 0.279  9.36   1.039  0.7318
##  Good - High       0.0532 0.267  8.82   0.199  0.9970
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 4 estimates
##
## [4] "\"for each increase of 1 unit pH_field, Bacterial_Functional_Diversity decreases 0.16
47 units (p=0.2151)\""
```

```
## [5] "\"for each increase of 1 unit Conductivity_field, Bacterial_Functional_Diversity incr
eases 0.4663 units (p=0.2521)\""

## Overall model p value for Overall_Status=0.565413886170973
```

6.2.2 Vertebrates

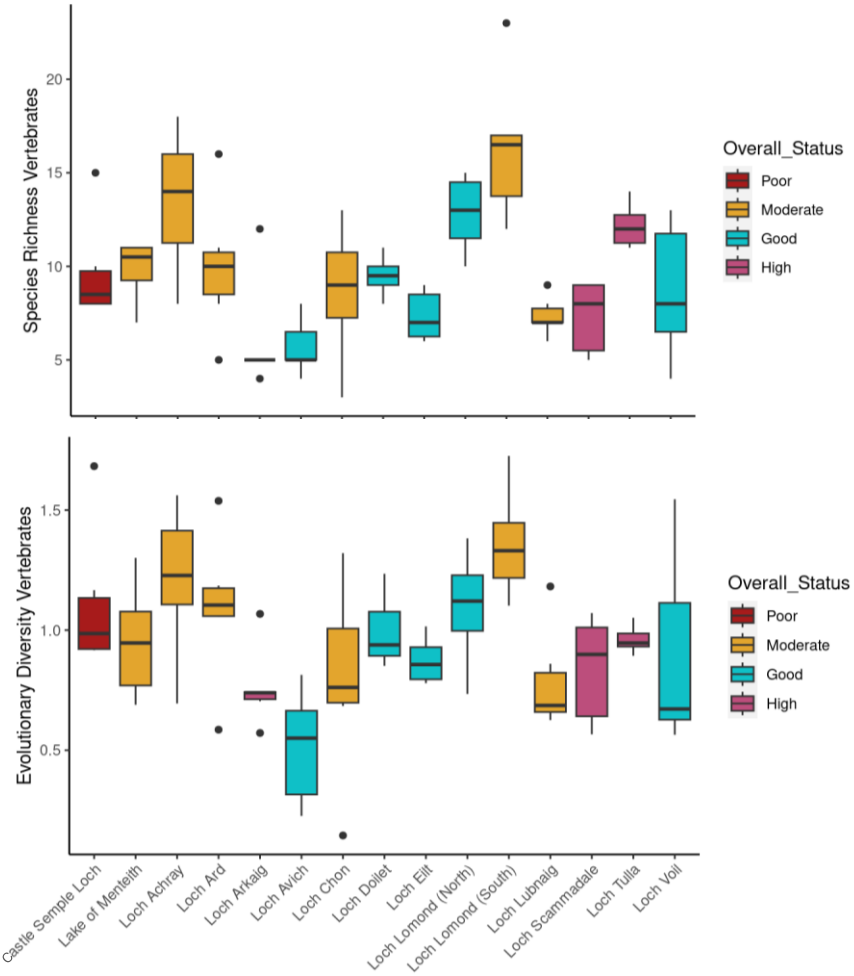


Figure 6: Medians and percentiles for Vertebrate sample-level metrics. Contains SEPA data © Scottish Environment Protection Agency and database right (2023). All rights reserved.

```
## Species_Richness_Vertebrates ~ Overall_Status + pH_field + Conductivity_field + (1 | Site)
## $`emmeans of Overall_Status`
##  Overall_Status emmean   SE   df lower.CL upper.CL
##  Poor             9.81 3.12 10.5     2.90     16.7
##  Moderate        11.20 1.29 10.9     8.37     14.0
##  Good             8.60 1.40 10.6     5.51     11.7
##  High             8.26 1.82 10.8     4.25     12.3
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Overall_Status`
##  1               estimate   SE   df t.ratio p.value
##  Poor - Moderate    -1.40 3.37 10.5  -0.415  0.9746
##  Poor - Good         1.20 3.42 10.6   0.352  0.9843
##  Poor - High         1.54 3.62 10.7   0.426  0.9728
##  Moderate - Good     2.60 1.91 11.0   1.359  0.5475
##  Moderate - High     2.94 2.25 11.2   1.306  0.5778
##  Good - High         0.34 2.28 10.6   0.149  0.9988


## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 4 estimates
##
## [4] "\"for each increase of 1 unit pH_field, Species_Richness_Vertebrates decreases 0.7684
units (p=0.3648)\""
## [5] "\"for each increase of 1 unit Conductivity_field, Species_Richness_Vertebrates increa
ses 4.2008 units (p=0.0532)\""
## Overall model p value for Overall_Status=0.497530609195422


## Evolutionary_Diversity_Vertebrates ~ Overall_Status + pH_field +
##     Conductivity_field + (1 | Site)
## $`emmeans of Overall_Status`
##  Overall_Status emmean     SE   df lower.CL upper.CL
##  Poor            1.120 0.2093 10.2    0.655     1.58
##  Moderate        1.052 0.0873 10.8    0.859     1.24
##  Good            0.858 0.0941 10.4    0.649     1.07
##  High            0.836 0.1230 10.7    0.564     1.11
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
```

```
## $`pairwise differences of Overall_Status`

##  1               estimate    SE   df t.ratio p.value

##  Poor - Moderate   0.0679 0.226 10.2   0.301  0.9900

##  Poor - Good       0.2618 0.230 10.3   1.138  0.6756

##  Poor - High       0.2834 0.244 10.4   1.161  0.6621

##  Moderate - Good   0.1939 0.130 10.9   1.493  0.4733

##  Moderate - High   0.2156 0.154 11.2   1.403  0.5225

##  Good - High       0.0216 0.153 10.3   0.141  0.9989

##

## Degrees-of-freedom method: kenward-roger

## P value adjustment: tukey method for comparing a family of 4 estimates

##

## [4] "\"for each increase of 1 unit pH_field, Evolutionary_Diversity_Vertebrates decreases
0.0572 units (p=0.4475)\""

## [5] "\"for each increase of 1 unit Conductivity_field, Evolutionary_Diversity_Vertebrates
increases 0.3279 units (p=0.1096)\""

## Overall model p value for Overall_Status=0.349735706897832
```

6.2.3 Freshwater Insects

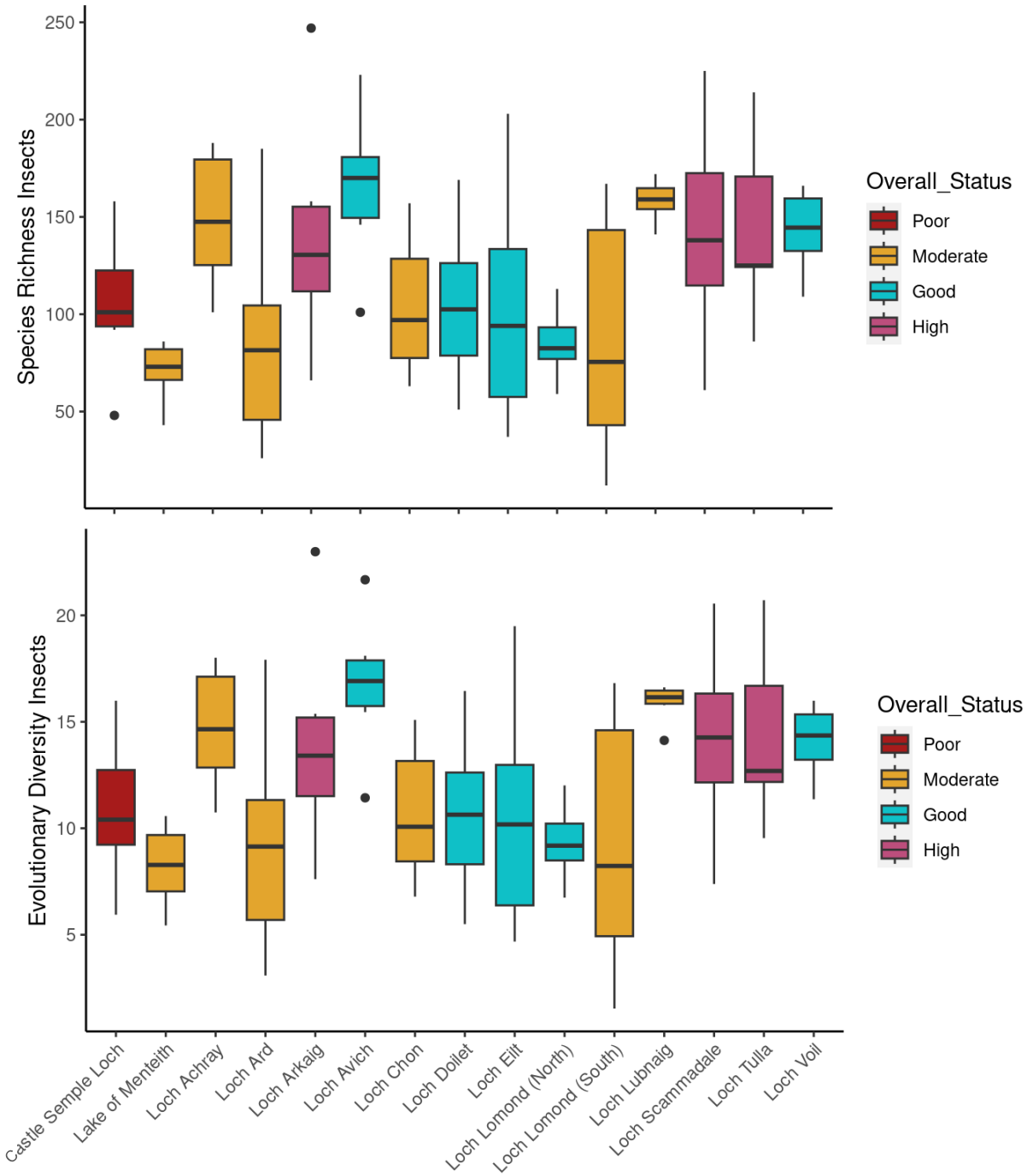


Figure 7: Medians and percentiles for Freshwater Insects sample-level metrics. Contains SEPA data © Scottish Environment Protection Agency and database right (2023). All rights reserved.

```
## Species_Richness_Insects ~ Overall_Status + pH_field + Conductivity_field +
##     (1 | Site)
## $`emmeans of Overall_Status`
##  Overall_Status emmean   SE   df lower.CL upper.CL
##  Poor              105 32.2 10.1     33.4      177
##  Moderate          110 13.5 10.7     80.5      140
##  Good              119 14.5 10.3     87.1      151
##  High              142 19.0 10.6     99.6      184
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Overall_Status`
##  1               estimate   SE   df t.ratio p.value
##  Poor - Moderate    -5.19 34.8 10.1  -0.149  0.9987
##  Poor - Good       -14.17 35.5 10.2  -0.400  0.9772
##  Poor - High       -36.57 37.6 10.3  -0.971  0.7682
##  Moderate - Good    -8.98 20.1 10.9  -0.447  0.9689
##  Moderate - High   -31.38 23.8 11.1  -1.317  0.5715
##  Good - High       -22.40 23.6 10.2  -0.948  0.7805
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 4 estimates
##
## [4] "\"for each increase of 1 unit pH_field, Species_Richness_Insects decreases 1.0063 uni
ts (p=0.9362)\""
## [5] "\"for each increase of 1 unit Conductivity_field, Species_Richness_Insects increases
31.6344 units (p=0.3669)\""
## Overall model p value for Overall_Status=0.598861623127089


## Evolutionary_Diversity_Insects ~ Overall_Status + pH_field +
##     Conductivity_field + (1 | Site)
## $`emmeans of Overall_Status`
##  Overall_Status emmean   SE   df lower.CL upper.CL
##  Poor             10.8 2.93 10.1     4.32     17.4
##  Moderate         11.4 1.22 10.7     8.67     14.1
##  Good             12.2 1.32 10.3     9.30     15.2
##  High             14.2 1.73 10.6    10.37     18.0
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
```

```
##
## $`pairwise differences of Overall_Status`
##  1               estimate   SE   df t.ratio p.value
##  Poor - Moderate   -0.537 3.16 10.1  -0.170  0.9981
##  Poor - Good       -1.389 3.22 10.2  -0.431  0.9717
##  Poor - High       -3.345 3.42 10.4  -0.978  0.7645
##  Moderate - Good   -0.852 1.82 10.9  -0.467  0.9647
##  Moderate - High   -2.808 2.16 11.2  -1.299  0.5820
##  Good - High       -1.956 2.15 10.2  -0.911  0.7994
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 4 estimates
##
## [4] "\"for each increase of 1 unit pH_field, Evolutionary_Diversity_Insects increases 0.11
67 units (p=0.9176)\""
## [5] "\"for each increase of 1 unit Conductivity field, Evolutionary Diversity Insects incr
eases 2.7139 units (p=0.3863)\""
## Overall model p value for Overall_Status=0.605564522392769
```

6.3 Woodland

6.3.1 Soil Invertebrates

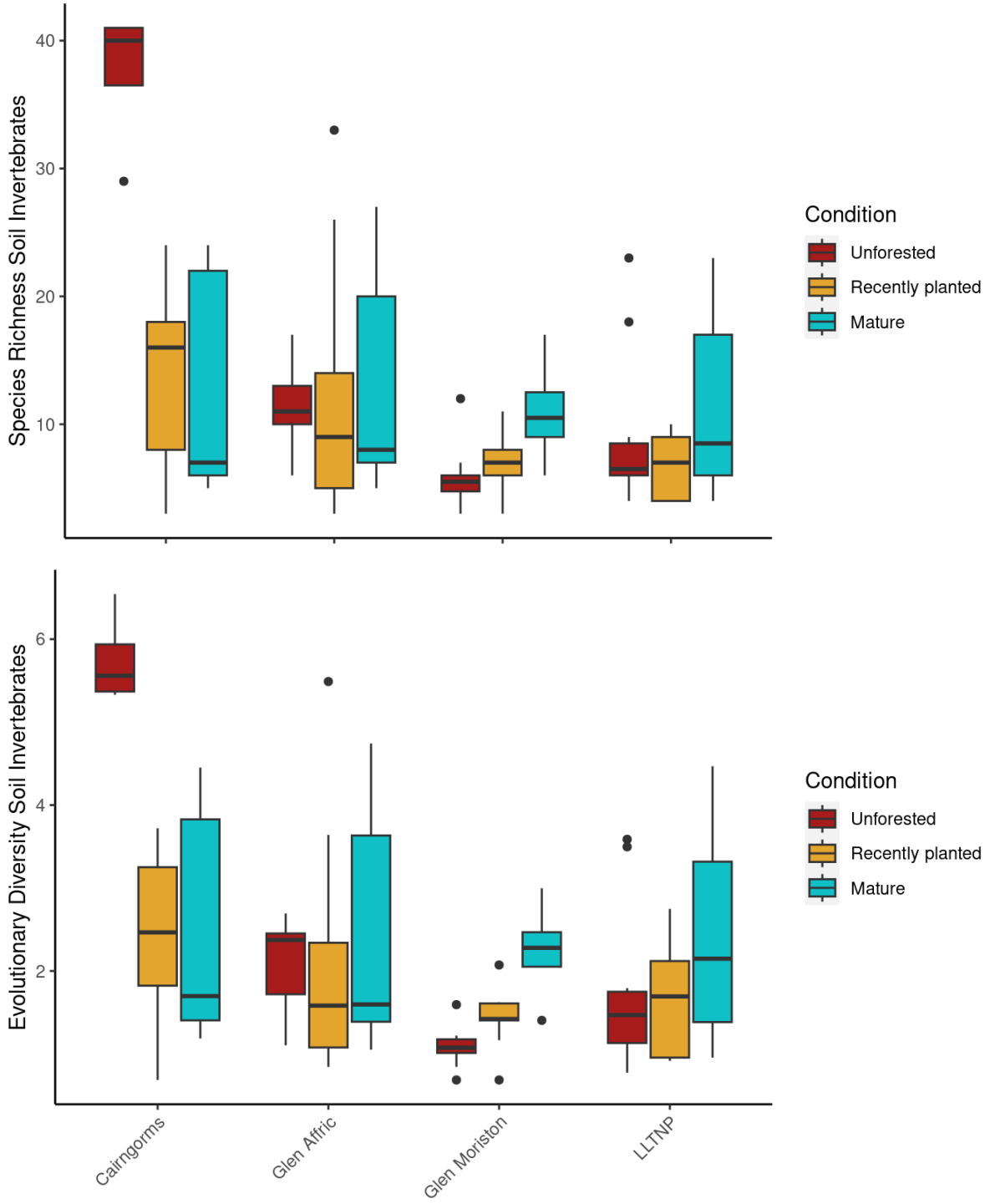


Figure 8: Medians and percentiles for Soil Invertebrates sample-level metrics.

```
## Species_Richness_Soil_Invertebrates ~ Condition + pH + Moisture +
##     (1 | Site)
## $`emmeans of Condition`
##  Condition        emmean   SE   df lower.CL upper.CL
##  Unforested         13.3 2.46 5.80     7.21     19.3
##  Recently planted   10.4 2.30 4.51     4.27     16.5
##  Mature             11.1 2.44 5.60     4.99     17.1
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Condition`
##  1                            estimate   SE   df t.ratio p.value
##  Unforested - Recently planted    2.910 1.94 90.1   1.498  0.2967
##  Unforested - Mature              2.220 2.45 90.7   0.908  0.6370
##  Recently planted - Mature       -0.691 2.01 90.3  -0.344  0.9371
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
##
## [3] "\"for each increase of 1 unit pH, Species_Richness_Soil_Invertebrates increases 3.634
4 units (p=0.0998)\""
## [4] "\"for each increase of 1 unit Moisture, Species_Richness_Soil_Invertebrates decreases
0.2062 units (p=1e-04)\""
## Overall model p value for Condition=0.326821069902751


## Evolutionary_Diversity_Soil_Invertebrates ~ Condition + pH +
##     Moisture + (1 | Site)
## $`emmeans of Condition`
##  Condition        emmean    SE   df lower.CL upper.CL
##  Unforested         2.29 0.352 6.38     1.44     3.13
##  Recently planted   1.97 0.326 4.82     1.12     2.81
##  Mature             2.21 0.348 6.13     1.36     3.05
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Condition`
##  1                            estimate    SE   df t.ratio p.value
##  Unforested - Recently planted    0.319 0.295 90.2   1.080  0.5286
##  Unforested - Mature              0.079 0.371 90.9   0.213  0.9753
##  Recently planted - Mature       -0.240 0.305 90.5  -0.785  0.7131
```

```
##

## Degrees-of-freedom method: kenward-roger

## P value adjustment: tukey method for comparing a family of 3 estimates

##

## [3] "\"for each increase of 1 unit pH, Evolutionary_Diversity_Soil_Invertebrates increases
0.4916 units (p=0.1416)\""

## [4] "\"for each increase of 1 unit Moisture, Evolutionary_Diversity_Soil_Invertebrates dec
reases 0.0348 units (p=0)\""

## Overall model p value for Condition=0.480316164993715
```

6.3.2 Fungi

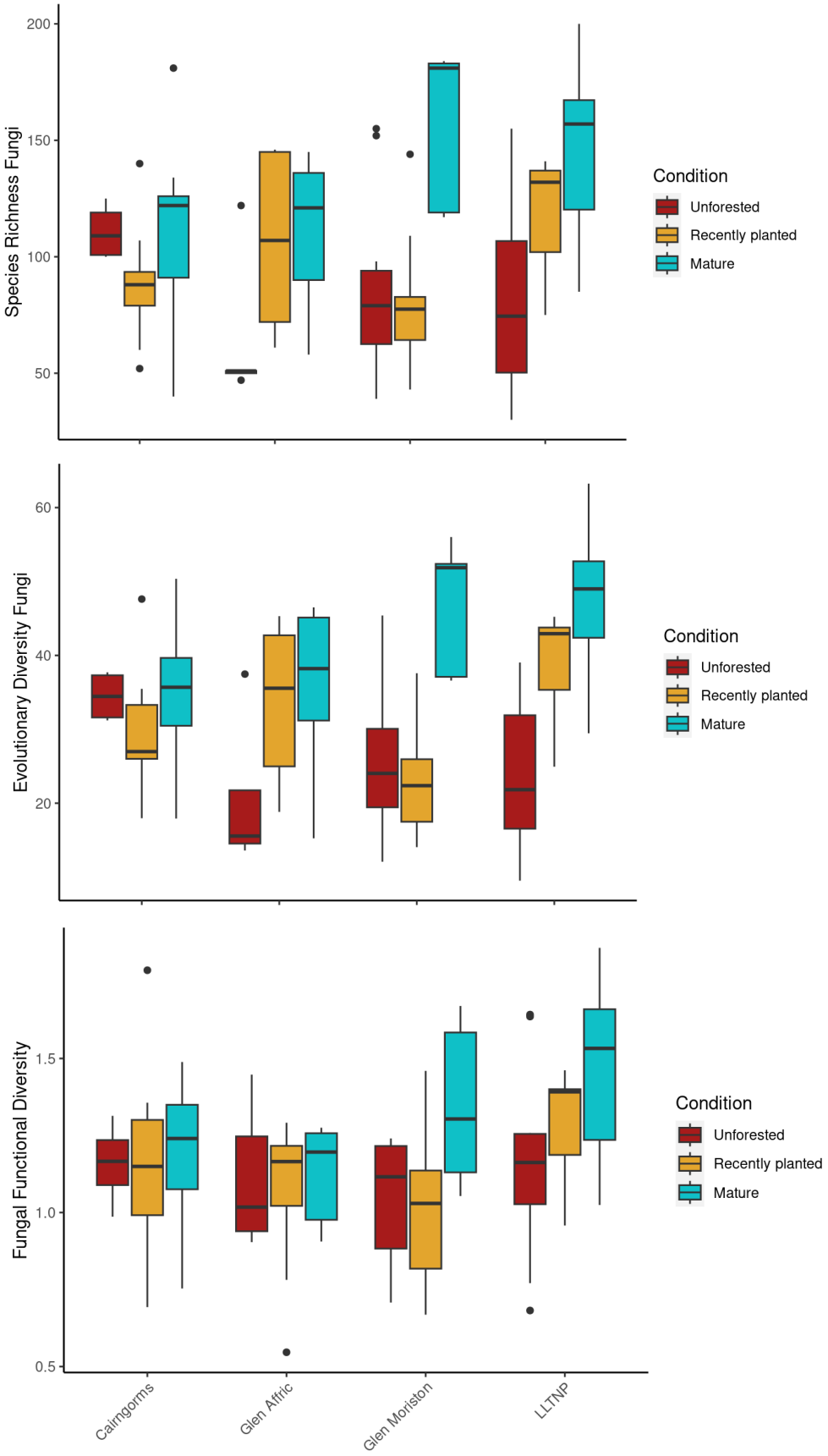


Figure 9: Medians and percentiles for Fungal sample-level metrics.

```
## Species_Richness_Fungi ~ Condition + pH + Moisture + (1 | Site)
## $`emmeans of Condition`
##  Condition        emmean   SE    df lower.CL upper.CL
##  Unforested         80.4 8.27 16.64     62.9     97.8
##  Recently planted   96.2 6.89  9.37     80.7    111.7
##  Mature            129.9 7.79 13.63    113.2    146.7
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Condition`
##  1                            estimate    SE   df t.ratio p.value
##  Unforested - Recently planted    -15.8  9.43 93.8  -1.674  0.2205
##  Unforested - Mature              -49.6 11.57 94.0  -4.283  0.0001
##  Recently planted - Mature        -33.8  9.38 94.0  -3.600  0.0015
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
##
## [3] "\"for each increase of 1 unit pH, Species_Richness_Fungi increases 16.1293 units (p=0
.119)\""
## [4] "\"for each increase of 1 unit Moisture, Species_Richness_Fungi decreases 0.5695 units
(p=0.0133)\""
## Overall model p value for Condition=8.52353380234338e-05


## Evolutionary_Diversity_Fungi ~ Condition + pH + Moisture + (1 |
##     Site)
## $`emmeans of Condition`
##  Condition        emmean   SE    df lower.CL upper.CL
##  Unforested         24.7 2.50 13.14     19.3     30.1
##  Recently planted   30.5 2.13  7.66     25.5     35.4
##  Mature             40.9 2.37 10.89     35.7     46.1
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Condition`
##  1                            estimate   SE   df t.ratio p.value
##  Unforested - Recently planted    -5.73 2.69 93.4  -2.127  0.0899
##  Unforested - Mature             -16.20 3.31 93.9  -4.893  <.0001
##  Recently planted - Mature       -10.47 2.68 93.9  -3.902  0.0005
##
## Degrees-of-freedom method: kenward-roger
```

```
## P value adjustment: tukey method for comparing a family of 3 estimates

##

## [3] "\"for each increase of 1 unit pH, Evolutionary_Diversity_Fungi increases 4.1431 units
(p=0.162)\""

## [4] "\"for each increase of 1 unit Moisture, Evolutionary_Diversity_Fungi decreases 0.1781
units (p=0.0078)\""

## Overall model p value for Condition=9.88413061183587e-06


## Fungal_Functional_Diversity ~ Condition + pH + Moisture + (1 |

##     Site)

## $`emmeans of Condition`

##  Condition        emmean     SE   df lower.CL upper.CL

##  Unforested         1.07 0.0693 9.04    0.913     1.23

##  Recently planted   1.12 0.0613 5.78    0.968     1.27

##  Mature             1.31 0.0666 7.73    1.156     1.47

##

## Degrees-of-freedom method: kenward-roger

## Confidence level used: 0.95

##

## $`pairwise differences of Condition`

##  1                            estimate     SE   df t.ratio p.value

##  Unforested - Recently planted  -0.0505 0.0668 92.7  -0.755  0.7313

##  Unforested - Mature            -0.2414 0.0822 93.3  -2.937  0.0115

##  Recently planted - Mature      -0.1909 0.0666 93.3  -2.867  0.0141

##

## Degrees-of-freedom method: kenward-roger

## P value adjustment: tukey method for comparing a family of 3 estimates

##

## [3] "\"for each increase of 1 unit pH, Fungal_Functional_Diversity increases 0.1345 units
(p=0.0695)\""

## [4] "\"for each increase of 1 unit Moisture, Fungal_Functional_Diversity decreases 0.0021
units (p=0.205)\""

## Overall model p value for Condition=0.00633737036639761
```

6.3.3 Bacteria

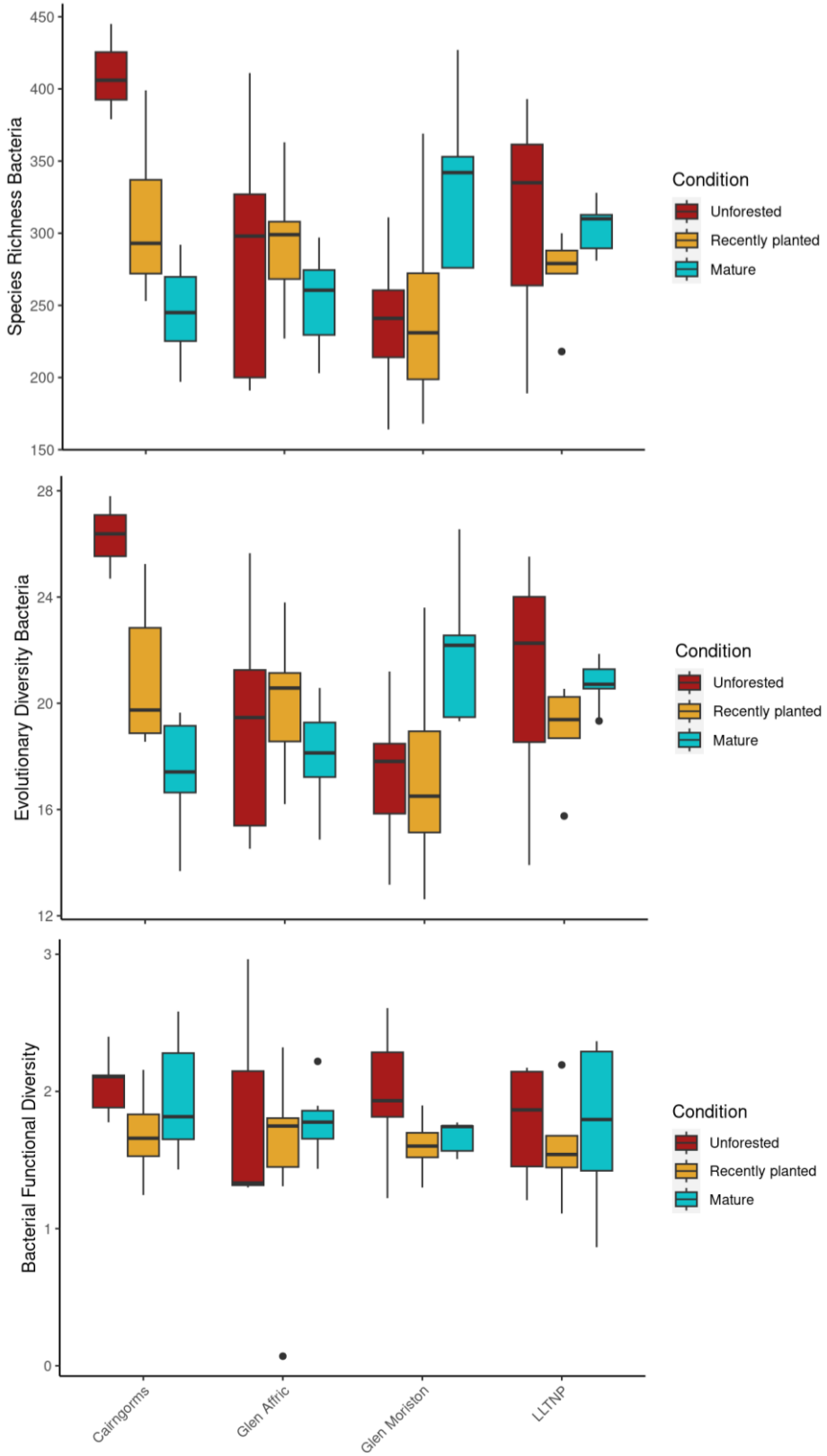


Figure 10: Medians and percentiles for Bacterial sample-level metrics.

```
## Species_Richness_Bacteria ~ Condition + pH + Moisture + (1 |
##     Site)
## $`emmeans of Condition`
##  Condition        emmean   SE   df lower.CL upper.CL
##  Unforested          272 15.8 9.26      237      308
##  Recently planted    277 13.7 5.53      243      311
##  Mature              293 14.8 7.19      258      328
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Condition`
##  1                            estimate   SE   df t.ratio p.value
##  Unforested - Recently planted    -4.48 14.8 94.0  -0.304  0.9504
##  Unforested - Mature             -20.47 18.3 94.2  -1.117  0.5057
##  Recently planted - Mature       -15.99 14.2 93.4  -1.130  0.4983
##
## Degrees-of-freedom method: kenward-roger
## P value adjustment: tukey method for comparing a family of 3 estimates
##
## [3] "\"for each increase of 1 unit pH, Species_Richness_Bacteria increases 72.7748 units (
p=0)\""
## [4] "\"for each increase of 1 unit Moisture, Species_Richness_Bacteria decreases 0.5914 un
its (p=0.1329)\""
## Overall model p value for Condition=0.454283577392559


## Evolutionary_Diversity_Bacteria ~ Condition + pH + Moisture +
##     (1 | Site)
## $`emmeans of Condition`
##  Condition        emmean    SE   df lower.CL upper.CL
##  Unforested         18.9 0.862 8.71     17.0     20.9
##  Recently planted   19.2 0.752 5.31     17.3     21.1
##  Mature             20.0 0.806 6.82     18.1     21.9
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Condition`
##  1                            estimate    SE   df t.ratio p.value
##  Unforested - Recently planted   -0.292 0.785 93.9  -0.372  0.9267
##  Unforested - Mature             -1.064 0.975 94.1  -1.092  0.5214
##  Recently planted - Mature       -0.772 0.753 93.3  -1.026  0.5625
##
```

```
## Degrees-of-freedom method: kenward-roger

## P value adjustment: tukey method for comparing a family of 3 estimates

##

## [3] "\"for each increase of 1 unit pH, Evolutionary_Diversity_Bacteria increases 3.8599 un
its (p=0)\""

## [4] "\"for each increase of 1 unit Moisture, Evolutionary_Diversity_Bacteria decreases 0.0
338 units (p=0.1078)\""

## Overall model p value for Condition=0.494672541365964


## Bacterial_Functional_Diversity ~ Condition + pH + Moisture +

##     (1 | Site)

## $`emmeans of Condition`

##  Condition        emmean     SE   df lower.CL upper.CL

##  Unforested         1.93 0.0918 22.1     1.74     2.12

##  Recently planted   1.61 0.0734 18.1     1.46     1.76

##  Mature             1.79 0.0801 21.5     1.62     1.96

##

## Degrees-of-freedom method: kenward-roger

## Confidence level used: 0.95

##

## $`pairwise differences of Condition`

##  1                            estimate    SE   df t.ratio p.value

##  Unforested - Recently planted    0.319 0.115 91.9   2.769  0.0185

##  Unforested - Mature              0.138 0.138 81.6   0.997  0.5811

##  Recently planted - Mature       -0.181 0.112 89.8  -1.616  0.2442

##

## Degrees-of-freedom method: kenward-roger

## P value adjustment: tukey method for comparing a family of 3 estimates

##

## [3] "\"for each increase of 1 unit pH, Bacterial_Functional_Diversity increases 0.0117 uni
ts (p=0.9216)\""

## [4] "\"for each increase of 1 unit Moisture, Bacterial_Functional_Diversity decreases 0.00
14 units (p=0.5759)\""

## Overall model p value for Condition=0.0130830267627195
```

6.4 Peatland

6.4.1 Soil Invertebrates

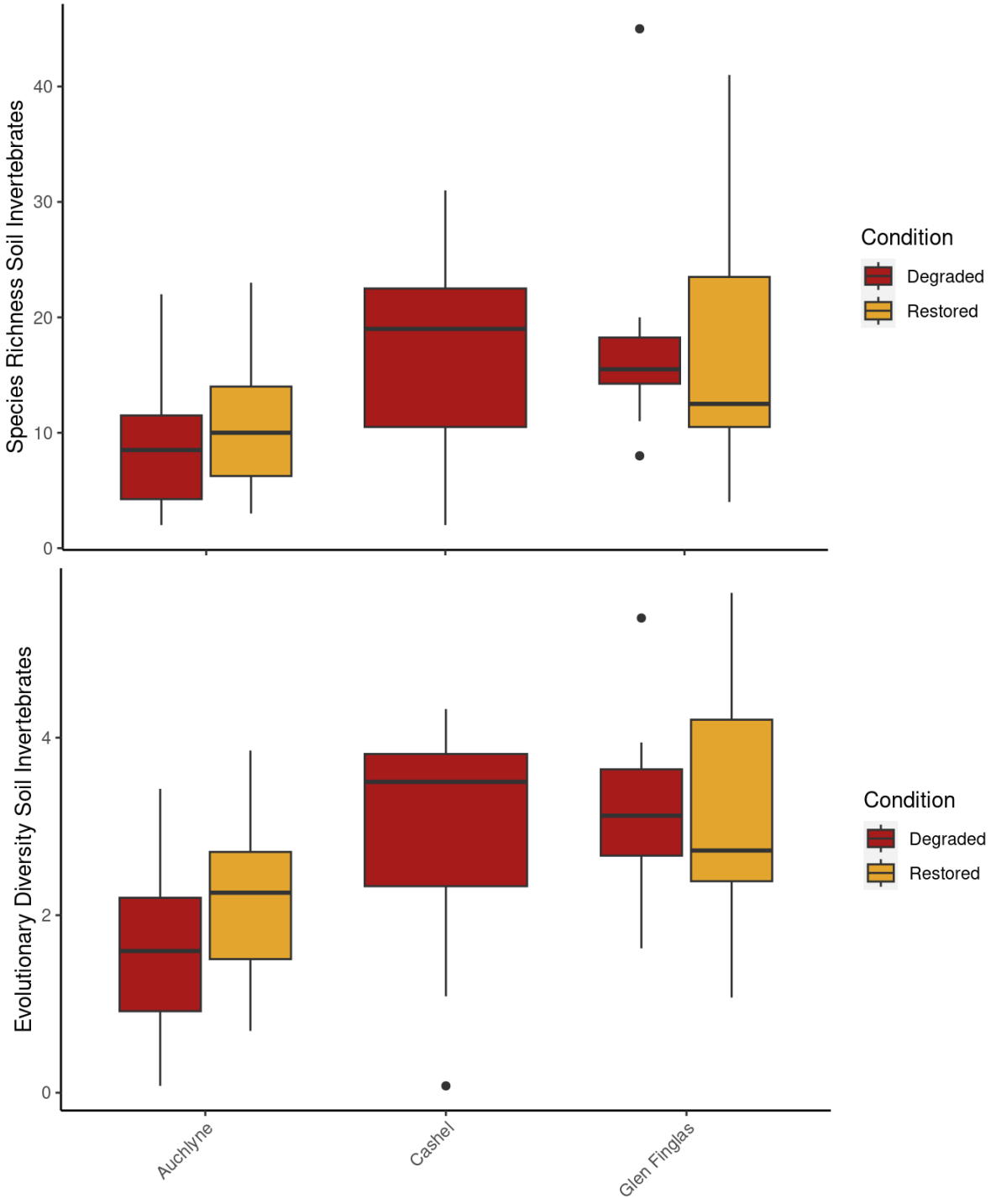


Figure 11: Medians and percentiles for Soil Invertebrates sample-level metrics.

```
## Species_Richness_Soil_Invertebrates ~ Condition + pH + Moisture +
##     (1 | Site)
## $`emmeans of Condition`
##  Condition emmean   SE   df lower.CL upper.CL
##  Degraded    14.6 2.51 2.55     5.79     23.5
##  Restored    14.9 3.18 3.82     5.87     23.9
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Condition`
##  1                   estimate   SE   df t.ratio p.value
##  Degraded - Restored   -0.212 3.18 43.2  -0.067  0.9470
##
## Degrees-of-freedom method: kenward-roger
##
## [2] "\"for each increase of 1 unit pH, Species_Richness_Soil_Invertebrates increases 3.705
4 units (p=0.2653)\""
## [3] "\"for each increase of 1 unit Moisture, Species_Richness_Soil_Invertebrates increases
0.2922 units (p=0.3892)\""
## Overall model p value for Condition=0.942710577625512


## Evolutionary_Diversity_Soil_Invertebrates ~ Condition + pH +
##     Moisture + (1 | Site)
## $`emmeans of Condition`
##  Condition emmean    SE   df lower.CL upper.CL
##  Degraded    2.63 0.403 2.32     1.11     4.15
##  Restored    2.74 0.476 3.43     1.33     4.16
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Condition`
##  1                   estimate    SE   df t.ratio p.value
##  Degraded - Restored   -0.114 0.417 45.4  -0.274  0.7857
##
## Degrees-of-freedom method: kenward-roger
##
## [2] "\"for each increase of 1 unit pH, Evolutionary_Diversity_Soil_Invertebrates increases
0.2598 units (p=0.5526)\""
## [3] "\"for each increase of 1 unit Moisture, Evolutionary_Diversity_Soil_Invertebrates inc
reases 0.0529 units (p=0.2407)\""
## Overall model p value for Condition=0.772994307029993
```

6.4.2 Fungi

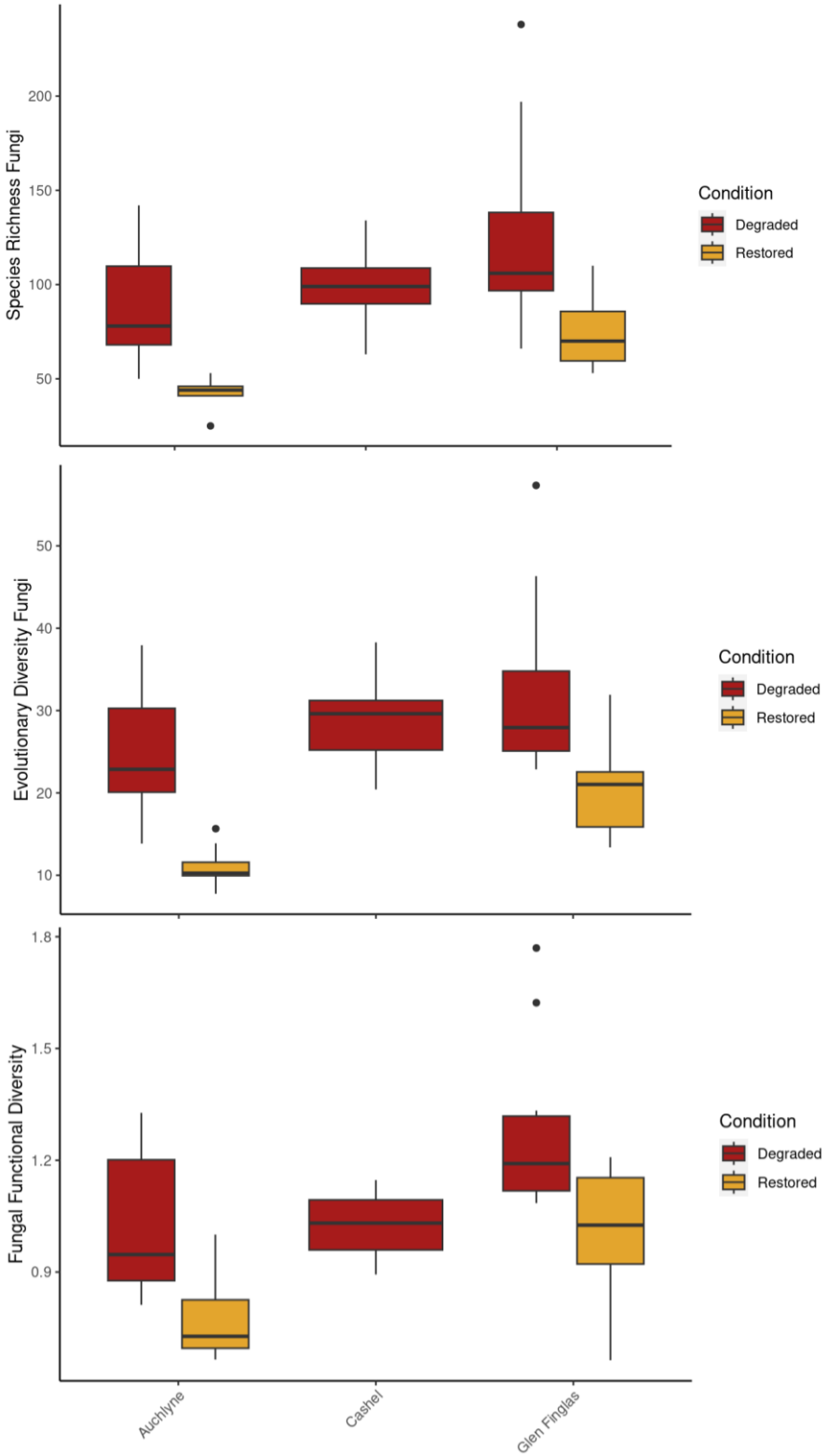


Figure 12: Medians and percentiles for Fungal sample-level metrics.

```
## Species_Richness_Fungi ~ Condition + pH + Moisture + (1 | Site)
## $`emmeans of Condition`
##  Condition emmean   SE   df lower.CL upper.CL
##  Degraded   102.9 10.2 2.28     63.6    142.1
##  Restored    60.6 11.9 3.30     24.7     96.5
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Condition`
##  1                   estimate   SE   df t.ratio p.value
##  Degraded - Restored     42.2 9.96 45.7   4.241  0.0001
##
## Degrees-of-freedom method: kenward-roger
##
## [2] "\"for each increase of 1 unit pH, Species_Richness_Fungi increases 18.9778 units (p=0
.0748)\""
## [3] "\"for each increase of 1 unit Moisture, Species_Richness_Fungi decreases 1.0506 units
(p=0.3301)\""
## Overall model p value for Condition=5.02504441449867e-05



## Evolutionary_Diversity_Fungi ~ Condition + pH + Moisture + (1 |
##     Site)
## $`emmeans of Condition`
##  Condition emmean   SE   df lower.CL upper.CL
##  Degraded    28.4 2.58 2.25    18.38     38.4
##  Restored    16.5 2.96 3.21     7.42     25.6
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Condition`
##  1                   estimate  SE   df t.ratio p.value
##  Degraded - Restored     11.9 2.4 45.9   4.955  <.0001
##
## Degrees-of-freedom method: kenward-roger
##
## [2] "\"for each increase of 1 unit pH, Evolutionary_Diversity_Fungi increases 2.9095 units
(p=0.2524)\""
## [3] "\"for each increase of 1 unit Moisture, Evolutionary_Diversity_Fungi decreases 0.1944
units (p=0.4542)\""
## Overall model p value for Condition=4.37656379491563e-06
```

```
## Fungal_Functional_Diversity ~ Condition + pH + Moisture + (1 |
##     Site)
## $`emmeans of Condition`
##  Condition emmean     SE   df lower.CL upper.CL
##  Degraded   1.104 0.0774 2.12    0.789     1.42
##  Restored   0.866 0.0872 3.08    0.592     1.14
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Condition`
##  1                   estimate    SE   df t.ratio p.value
##  Degraded - Restored    0.238 0.062 42.9   3.842  0.0004
##
## Degrees-of-freedom method: kenward-roger
##
## [2] "\"for each increase of 1 unit pH, Fungal_Functional_Diversity increases 0.1165 units
(p=0.0774)\""
## [3] "\"for each increase of 1 unit Moisture, Fungal_Functional_Diversity increases 4e-04 u
nits (p=0.9511)\""
## Overall model p value for Condition=0.00026561361257618
```

6.4.3 Bacteria

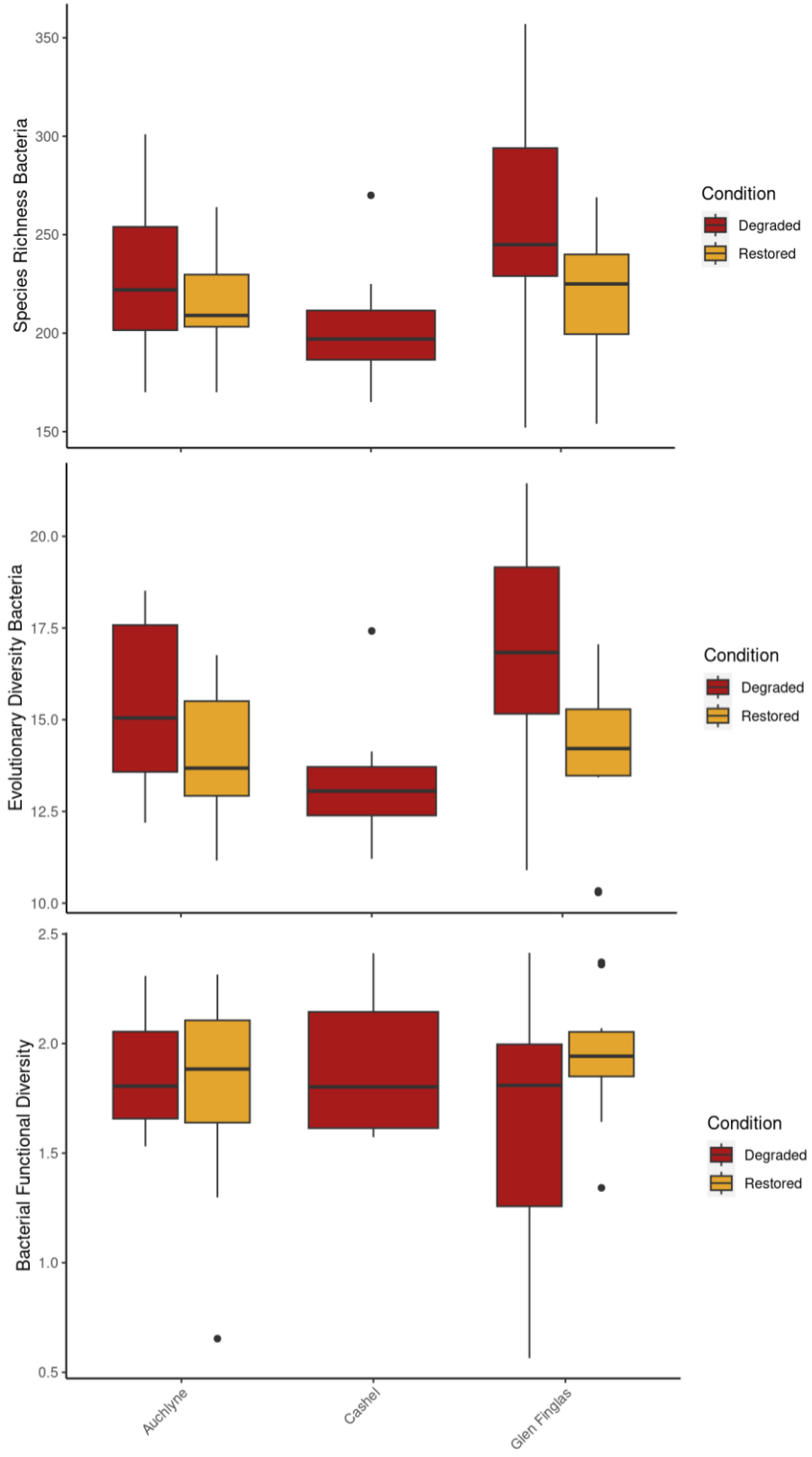


Figure 13: Medians and percentiles for Bacterial sample-level metrics.

```
## Species_Richness_Bacteria ~ Condition + pH + Moisture + (1 |
##     Site)
## $`emmeans of Condition`
##  Condition emmean   SE   df lower.CL upper.CL
##  Degraded     228 14.6 2.22      171      285
##  Restored     208 16.4 3.04      157      260
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Condition`
##  1                   estimate   SE df t.ratio p.value
##  Degraded - Restored     19.5 12.6 45   1.545  0.1294
##
## Degrees-of-freedom method: kenward-roger
##
## [2] "\"for each increase of 1 unit pH, Species_Richness_Bacteria increases 50.345 units (p
=4e-04)\""
## [3] "\"for each increase of 1 unit Moisture, Species_Richness_Bacteria decreases 0.6457 un
its (p=0.6338)\""
## Overall model p value for Condition=0.112650685729257


## Evolutionary_Diversity_Bacteria ~ Condition + pH + Moisture +
##     (1 | Site)
## $`emmeans of Condition`
##  Condition emmean    SE   df lower.CL upper.CL
##  Degraded    15.0 0.932 2.17     11.3     18.8
##  Restored    13.5 1.020 2.83     10.1     16.8
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Condition`
##  1                   estimate    SE df t.ratio p.value
##  Degraded - Restored     1.56 0.707 45   2.203  0.0328
##
## Degrees-of-freedom method: kenward-roger
##
## [2] "\"for each increase of 1 unit pH, Evolutionary_Diversity_Bacteria increases 2.8291 un
its (p=5e-04)\""
## [3] "\"for each increase of 1 unit Moisture, Evolutionary_Diversity_Bacteria decreases 0.0
597 units (p=0.4353)\""
## Overall model p value for Condition=0.0270071868165954
```

```
## Fungal_Functional_Diversity ~ Condition + pH + Moisture + (1 |
##     Site)
## $`emmeans of Condition`
##  Condition emmean     SE   df lower.CL upper.CL
##  Degraded   1.104 0.0774 2.12    0.789     1.42
##  Restored   0.866 0.0872 3.08    0.592     1.14
##
## Degrees-of-freedom method: kenward-roger
## Confidence level used: 0.95
##
## $`pairwise differences of Condition`
##  1                   estimate    SE   df t.ratio p.value
##  Degraded - Restored    0.238 0.062 42.9   3.842  0.0004
##
## Degrees-of-freedom method: kenward-roger
##
## [2] "\"for each increase of 1 unit pH, Fungal_Functional_Diversity increases 0.1165 units
(p=0.0774)\""
## [3] "\"for each increase of 1 unit Moisture, Fungal_Functional_Diversity increases 4e-04 u
nits (p=0.9511)\""
## Overall model p value for Condition=0.00026561361257618
```

7 Random Forest Classification Tables

This Section provides the results of the cross-validation tests of predictive classification using Random Forest.

7.1 Marine

Table 2: Cross-validation tests of predictive classification accuracy using Random Forest for Level 5 Biotopes. Each table reports one assay.

		Correctly classified	Incorrectly classified	% correctly classified
SS.SMx.CMx.KurThyMx	LL13	0	3	0.0 %
	LL26	0	3	0.0 %
	LL32	0	3	0.0 %
	LL36	1	2	33.3 %
	LL54	1	1	50.0 %
	Total	2	12	14.3 %
SS.SSa.CMuSa.AalbNuc	LL07	2	1	66.7 %
	LL11	3	0	100.0 %
	LL14	3	0	100.0 %
	LL16	3	0	100.0 %
	LL17	1	2	33.3 %
	LL30	3	0	100.0 %
	LL33	2	1	66.7 %
	LL43	1	2	33.3 %
	LL46	3	0	100.0 %
	LL50	3	0	100.0 %
	Total	24	6	80.0 %
Total		26	18	59.1 %

Marine – sediment bacteria

		Correctly classified	Incorrectly classified	% correctly classified
SS.SMx.CMx.KurThyMx	LL13	0	3	0.0 %
	LL26	0	3	0.0 %
	LL32	0	3	0.0 %
	LL36	0	3	0.0 %
	LL54	0	2	0.0 %
	Total	0	14	0.0 %
SS.SSa.CMuSa.AalbNuc	LL07	2	1	66.7 %
	LL11	3	0	100.0 %
	LL14	3	0	100.0 %
	LL16	3	0	100.0 %
	LL17	1	2	33.3 %
	LL30	3	0	100.0 %
	LL33	2	1	66.7 %
	LL43	2	1	66.7 %
	LL46	3	0	100.0 %
	LL50	3	0	100.0 %
	Total	25	5	83.3 %
Total		25	19	56.8 %

Marine – sediment eukaryotes

		Correctly classified	Incorrectly classified	% correctly classified
SS.SMx.CMx.KurThyMx	LL13	0	3	0.0 %
	LL26	0	3	0.0 %
	LL32	0	3	0.0 %
	LL36	1	2	33.3 %
	LL54	0	2	0.0 %
	Total	1	13	7.1 %
SS.SSa.CMuSa.AalbNuc	LL07	3	0	100.0 %
	LL11	3	0	100.0 %
	LL14	3	0	100.0 %
	LL16	3	0	100.0 %
	LL17	2	1	66.7 %
	LL30	3	0	100.0 %
	LL33	3	0	100.0 %
	LL43	0	3	0.0 %
	LL46	3	0	100.0 %
	LL50	3	0	100.0 %
	Total	26	4	86.7 %
Total		27	17	61.4 %

Marine – sediment invertebrates

		Correctly classified	Incorrectly classified	% correctly classified
SS.SMx.CMx.KurThyMx	LL13	0	3	0.0 %
	LL26	1	2	33.3 %
	LL30	0	2	0.0 %
	LL33	0	2	0.0 %
	LL54	0	2	0.0 %
	Total	1	11	8.3 %
SS.SSa.CMuSa.AalbNuc	LL07	2	0	100.0 %
	LL11	1	1	50.0 %
	LL14	2	0	100.0 %
	LL16	1	1	50.0 %
	LL17	3	0	100.0 %
	LL28	2	0	100.0 %
	LL32	1	0	100.0 %
	LL36	2	0	100.0 %
	LL43	0	2	0.0 %
	LL46	2	0	100.0 %
	LL50	2	0	100.0 %
	Total	18	4	81.8 %
Total		19	15	55.9 %

Marine – fish

Table 3: Cross-validation tests of predictive classification accuracy using Random Forest Level 4 Biotopes. Each table reports one assay.

		Correctly classified	Incorrectly classified	% correctly classified
SS.SMu.CFiMu	LL07	2	1	66.7 %
	LL11	3	0	100.0 %
	LL14	3	0	100.0 %
	LL16	3	0	100.0 %
	LL30	3	0	100.0 %
	LL33	2	1	66.7 %
	LL43	1	2	33.3 %
	LL46	3	0	100.0 %
	LL50	3	0	100.0 %
	LL65	3	0	100.0 %
	Total	26	4	86.7 %
SS.SMu.CSaMu	LL25	0	3	0.0 %
	LL28	0	3	0.0 %
	Total	0	6	0.0 %
SS.SMx.CMx	LL03	3	0	100.0 %
	LL13	3	0	100.0 %
	LL17	2	1	66.7 %
	LL26	3	0	100.0 %
	LL32	0	3	0.0 %
	LL36	1	2	33.3 %
	LL54	0	2	0.0 %
	Total	12	8	60.0 %
Total		38	18	67.9 %

Marine – sediment bacteria

		Correctly classified	Incorrectly classified	% correctly classified
SS.SMu.CFiMu	LL07	2	1	66.7 %
	LL11	3	0	100.0 %
	LL14	3	0	100.0 %
	LL16	3	0	100.0 %
	LL30	3	0	100.0 %
	LL33	2	1	66.7 %
	LL43	2	1	66.7 %
	LL46	3	0	100.0 %
	LL50	3	0	100.0 %
	LL65	3	0	100.0 %
	Total	27	3	90.0 %
SS.SMu.CSaMu	LL25	0	3	0.0 %
	LL28	0	3	0.0 %
	Total	0	6	0.0 %
SS.SMx.CMx	LL03	3	0	100.0 %
	LL13	2	1	66.7 %
	LL17	2	1	66.7 %
	LL26	0	3	0.0 %
	LL32	0	3	0.0 %
	LL36	0	3	0.0 %
	LL54	2	0	100.0 %
	Total	9	11	45.0 %
Total		36	20	64.3 %

Marine – sediment eukaryotes

		Correctly classified	Incorrectly classified	% correctly classified
SS.SMu.CFiMu	LL07	2	1	66.7 %
	LL11	2	1	66.7 %
	LL14	2	1	66.7 %
	LL16	3	0	100.0 %
	LL30	3	0	100.0 %
	LL33	1	2	33.3 %
	LL43	0	3	0.0 %
	LL46	3	0	100.0 %
	LL50	3	0	100.0 %
	LL65	3	0	100.0 %
	Total	22	8	73.3 %
SS.SMu.CSaMu	LL25	0	3	0.0 %
	LL28	0	3	0.0 %
	Total	0	6	0.0 %
SS.SMx.CMx	LL03	3	0	100.0 %
	LL13	1	2	33.3 %
	LL17	1	2	33.3 %
	LL26	0	3	0.0 %
	LL32	0	3	0.0 %
	LL36	1	2	33.3 %
	LL54	2	0	100.0 %
	Total	8	12	40.0 %
Total		30	26	53.6 %

Marine – sediment invertebrates

		Correctly classified	Incorrectly classified	% correctly classified
SS.SMu.CFiMu	LL07	2	0	100.0 %
	LL11	1	1	50.0 %
	LL14	2	0	100.0 %
	LL16	1	1	50.0 %
	LL30	2	0	100.0 %
	LL33	2	0	100.0 %
	LL43	0	2	0.0 %
	LL46	2	0	100.0 %
	LL50	1	1	50.0 %
	LL65	2	0	100.0 %
	Total	15	5	75.0 %
SS.SMu.CSaMu	LL25	0	3	0.0 %
	LL28	0	2	0.0 %
	Total	0	5	0.0 %
SS.SMx.CMx	LL03	1	1	50.0 %
	LL13	0	3	0.0 %
	LL17	1	2	33.3 %
	LL26	1	2	33.3 %
	LL32	0	1	0.0 %
	LL36	0	2	0.0 %
	LL54	1	1	50.0 %
	Total	4	12	25.0 %
Total		19	22	46.3 %

Marine – fish

7.2 Freshwater

Table 4: Statistical output from the constrained ordination plot for fish communities.

	Estimate	Std. Error	z value	Pr(>|z|)
Overall.Status.GroupedModerate_Poor(CLV1)	0.675	0.477	1.416	0.157
PC1(CLV1)	-0.385	0.221	-1.743	0.081
Overall.Status.GroupedModerate_Poor(CLV2)	0.094	0.180	0.523	0.601
PC1(CLV2)	0.165	0.087	1.889	0.059

R^2 for latent variables: 0.766
Partial R^2 for predictors and all LVs:
Overall.Status.GroupedModerate_Poor PC1
0.660 0.035

Table 5: Statistical output from the constrained ordination plot for freshwater invertebrate communities.

Freshwater invertebrates	Estimate	Std. Error	z value	Pr(>|z|)
Overall.Status.GroupedModerate_Poor(CLV1)	0.120	0.863	0.139	0.889
PC1(CLV1) Moor <-> Urban/Woodland	-1.384	0.501	-2.760	0.006
Conductivity..mS.cm.(CLV1)	-0.836	0.351	-2.379	0.017
Overall.Status.GroupedModerate_Poor(CLV2)	0.088	0.008	10.719	0.000
PC1(CLV2)	0.005	0.004	1.262	0.207
Conductivity..mS.cm.(CLV2)	0.005	0.003	1.462	0.144

R^2 for latent variables: 0.464
Partial R^2 for predictors and all LVs:
Overall.Status.GroupedModerate_Poor PC1
0.411 0.055
Conductivity..mS.cm.
0.045

Table 6: Statistical output from the constrained ordination plot for bacteria communities.

Freshwater bacteria	Estimate	Std. Error	z value	Pr(>|z|)
Overall.Status.GroupedModerate_Poor(CLV1)	0.12	0.86	0.14	0.889
PC1(CLV1) Moor <-> Urban/Woodland	-1.38	0.50	-2.76	0.006
Conductivity..mS.cm.(CLV1)	-0.84	0.35	-2.38	0.017
Overall.Status.GroupedModerate_Poor(CLV2)	0.09	0.01	10.72	0.000
PC1(CLV2)	0.00	0.00	1.26	0.207
Conductivity..mS.cm.(CLV2)	0.00	0.00	1.46	0.144

R^2 for latent variables: 0.1876
Partial R^2 for predictors and all LVs:
Overall.Status.GroupedModerate_ PC1
-0.0917 0.1458
PC2 Conductivity..mS.cm.
0.2201 0.0227

Table 7: Cross-validation tests of predictive classification accuracy using Random Forest. Each table reports one assay.

		Correctly classified	Incorrectly classified	% correctly classified
High_Good	Loch Arkaig	4	2	66.7 %
	Loch Avich	5	1	83.3 %
	Loch Doilet	6	0	100.0 %
	Loch Eilt	3	3	50.0 %
	Loch Lomond (North)	0	6	0.0 %
	Loch Scammadale	6	0	100.0 %
	Loch Tulla	6	0	100.0 %
	Loch Voil	0	6	0.0 %
	Total	30	18	62.5 %
Moderate_Poor	Castle Semple Loch	6	0	100.0 %
	Lake of Menteith	4	2	66.7 %
	Loch Achray	4	2	66.7 %
	Loch Ard	6	0	100.0 %
	Loch Chon	0	6	0.0 %
	Loch Lomond (South)	1	5	16.7 %
	Loch Lubnaig	2	4	33.3 %
	Total	23	19	54.8 %
Total		53	37	58.9 %

Freshwater – bacteria

		Correctly classified	Incorrectly classified	% correctly classified
High_Good	Loch Arkaig	6	0	100.0 %
	Loch Avich	6	0	100.0 %
	Loch Doilet	6	0	100.0 %
	Loch Eilt	5	1	83.3 %
	Loch Lomond (North)	0	6	0.0 %
	Loch Scammadale	6	0	100.0 %
	Loch Tulla	2	4	33.3 %
	Loch Voil	4	2	66.7 %
	Total	35	13	72.9 %
Moderate_Poor	Castle Semple Loch	6	0	100.0 %
	Lake of Menteith	6	0	100.0 %
	Loch Achray	6	0	100.0 %
	Loch Ard	5	1	83.3 %
	Loch Chon	6	0	100.0 %
	Loch Lomond (South)	1	5	16.7 %
	Loch Lubnaig	0	6	0.0 %
	Total	30	12	71.4 %
Total		65	25	72.2 %

Freshwater – fish

		Correctly classified	Incorrectly classified	% correctly classified
High_Good	Loch Arkaig	6	0	100.0 %
	Loch Avich	5	1	83.3 %
	Loch Doilet	6	0	100.0 %
	Loch Eilt	6	0	100.0 %
	Loch Lomond (North)	1	5	16.7 %
	Loch Scammadale	5	1	83.3 %
	Loch Tulla	6	0	100.0 %
	Loch Voil	4	2	66.7 %
	Total	39	9	81.2 %
Moderate_Poor	Castle Semple Loch	6	0	100.0 %
	Lake of Menteith	6	0	100.0 %
	Loch Achray	4	2	66.7 %
	Loch Ard	6	0	100.0 %
	Loch Chon	3	3	50.0 %
	Loch Lomond (South)	4	2	66.7 %
	Loch Lubnaig	1	5	16.7 %
	Total	30	12	71.4 %
Total		69	21	76.7 %

Freshwater – invertebrates

7.3 Woodland

Table 8: Cross-validation tests of predictive classification accuracy using Random Forest. Each table reports one assay.

		Correctly classified	Incorrectly classified	% correctly classified
Unforested	Coille Coire Chuilc	5	0	100.0 %
	Coille Ruigh	3	0	100.0 %
	Dundreggan Allt Fearna	5	0	100.0 %
	Dundreggan WGS	4	2	66.7 %
	Ghubhais	1	1	50.0 %
	Glen Falloch	5	0	100.0 %
	Total	23	3	88.5 %
Recently planted	Coille Ruigh	2	3	40.0 %
	Dundreggan Allt Fearna	5	0	100.0 %
	Dundreggan WGS	3	2	60.0 %
	Ghubhais	4	1	80.0 %
	Glen Falloch	4	1	80.0 %
	Glenmore	0	5	0.0 %
	Rothiemurchas	0	4	0.0 %
	Total	18	16	52.9 %
Mature	Coille Coire Chuilc	3	3	50.0 %
	Coille Ruigh	5	0	100.0 %
	Ghubhais	2	3	40.0 %
	Glen Falloch	6	0	100.0 %
	Glenmore	5	0	100.0 %
	Inverwick	4	1	80.0 %
	Rothiemurchas	4	1	80.0 %
	Total	29	8	78.4 %
Total		70	27	72.2 %

Woodland – soil bacteria

		Correctly classified	Incorrectly classified	% correctly classified
Unforested	Coille Coire Chuilc	5	0	100.0 %
	Coille Ruigh	3	0	100.0 %
	Dundreggan Allt Fearna	4	2	66.7 %
	Dundreggan WGS	6	0	100.0 %
	Ghubhais	2	0	100.0 %
	Glen Falloch	5	0	100.0 %
	Total	25	2	92.6 %
Recently planted	Coille Ruigh	2	2	50.0 %
	Dundreggan Allt Fearna	0	5	0.0 %
	Dundreggan WGS	0	4	0.0 %
	Ghubhais	0	5	0.0 %
	Glen Falloch	2	3	40.0 %
	Glenmore	3	2	60.0 %
	Rothiemurchas	0	4	0.0 %
	Total	7	25	21.9 %
Mature	Coille Coire Chuilc	0	6	0.0 %
	Coille Ruigh	4	0	100.0 %
	Ghubhais	0	5	0.0 %
	Glen Falloch	4	2	66.7 %
	Glenmore	4	1	80.0 %
	Inverwick	3	1	75.0 %
	Rothiemurchas	4	0	100.0 %
	Total	19	15	55.9 %
Total		51	42	54.8 %

Woodland – soil invertebrates

		Correctly classified	Incorrectly classified	% correctly classified
Unforested	Coille Ruigh	3	0	100.0 %
	Dundreggan Allt Fearna	4	1	80.0 %
	Dundreggan WGS	2	4	33.3 %
	Ghubhais	1	1	50.0 %
	Total	10	6	62.5 %
Recently planted	Coille Ruigh	3	2	60.0 %
	Dundreggan Allt Fearna	5	0	100.0 %
	Dundreggan WGS	2	3	40.0 %
	Ghubhais	2	2	50.0 %
	Glenmore	1	4	20.0 %
	Rothiemurchas	5	0	100.0 %
	Total	18	11	62.1 %
Mature	Coille Ruigh	3	1	75.0 %
	Ghubhais	3	2	60.0 %
	Glenmore	4	0	100.0 %
	Inverwick	5	0	100.0 %
	Rothiemurchas	4	1	80.0 %
	Total	19	4	82.6 %
Total		47	21	69.1 %

Woodland – soil fungi

Table 9: Condition and Area are both significant terms for explaining variation along CLV1 (yellow and blue highlighting respectively).

	Estimate	Std. Error	z value	Pr(>|z|)
ConditionRecently planted(CLV1)	1.65341363	0.69391489	2.3827326	0.0171846713
ConditionMature(CLV1)	3.85365416	1.15876637	3.3256524	0.0008821186
AreaGlen Affric(CLV1)	2.18681659	0.74533511	-2.9340045	0.0033461932
AreaGlen Moriston(CLV1)	2.92684661	0.92931529	-3.1494657	0.0016356931
Moisture(CLV1)	0.05469715	0.27953797	-0.1956698	0.8448686006
ConditionRecently planted(CLV2)	0.07270348	0.18103210	-0.4016055	0.6879743943
ConditionMature(CLV2)	0.07946056	0.28690471	-0.2769580	0.7818123534
AreaGlen Affric(CLV2)	0.10814597	0.10028265	-1.0784116	0.2808501180
AreaGlen Moriston(CLV2)	0.06708416	0.09431662	-0.7112655	0.4769197221
Moisture(CLV2)	0.09597152	0.08589613	1.1172975	0.2638671643

R^2 for latent variables: 0.0777

Partial R^2 for predictors and all LVs:

ConditionRecently planted	ConditionMature	AreaGlen Affric
0.0230	0.0485	0.1066
AreaGlen Moriston	Moisture	
0.0759	-0.0032	

7.4 Peatland

Table 10: Cross-validation tests of predictive classification accuracy using Random Forest. Each table reports one assay.

		Correctly classified	Incorrectly classified	% correctly classified
Degraded	Auchlyne	8	2	80.0 %
	Cashel	3	7	30.0 %
	Glen Finglas	7	2	77.8 %
	Total	18	11	62.1 %
Restored	Auchlyne	5	5	50.0 %
	Glen Finglas	0	10	0.0 %
	Total	5	15	25.0 %
Total		23	26	46.9 %

Peatland – bacteria

		Correctly classified	Incorrectly classified	% correctly classified
Degraded	Auchlyne	7	3	70.0 %
	Cashel	7	3	70.0 %
	Glen Finglas	9	1	90.0 %
	Total	23	7	76.7 %
Restored	Auchlyne	9	1	90.0 %
	Glen Finglas	0	10	0.0 %
	Total	9	11	45.0 %
Total		32	18	64.0 %

Peatland – fungi

		Correctly classified	Incorrectly classified	% correctly classified
Degraded	Auchlyne	9	1	90.0 %
	Cashel	6	4	60.0 %
	Glen Finglas	7	3	70.0 %
	Total	22	8	73.3 %
Restored	Auchlyne	1	9	10.0 %
	Glen Finglas	2	8	20.0 %
	Total	3	17	15.0 %
Total		25	25	50.0 %

Peatland – soil invertebrates

	Estimate	Std. Error	z value	Pr(>|z|)
ConditionRestored(CLV1)	-0.00008983822	0.022243754	0.004038807	0.9967775074
Moisture(CLV1)	-0.02065030985	0.005961297	3.464063351	0.0005320812
pH(CLV1)	-0.00252918281	0.004415774	0.572760964	0.5668065533
ConditionRestored(CLV2)	-0.96137169618	0.926336119	1.037821668	0.2993530872
Moisture(CLV2)	-0.00415582524	0.020650187	0.201248793	0.8405040408
pH(CLV2)	0.06808298215	0.020288766	3.355698475	0.0007916483

[1] "From van Veen et al. (2022) The proportion of (generalized) variance explained by all p predictors and all d latent variables is"

R^2 for latent variables:0.2387

Partial $R^2$for predictors and all LVs:

ConditionRestored	Moisture	pH
0.0285	0.1146	0.1180

8 Conventional Marine Results

8.1 Description of Marine Biotope Classification Data

Morphoanalysis was conducted for 20 samples (one from each station) by SEPA, using the marine invertebrates collected during the project (see main report for methods). Using the output data, the biotope for each sample was classified (see main report for methods). Below we present further details of the classification for each station. Marine morphological (benthic invertebrates) and PSD data is not included in this report but can be requested from SEPA for a different purpose.

LL3 was defined as "*Cerianthus lloydii* and other burrowing anemones in circalittoral muddy mixed sediment" (SS.SMx.CMX.ClloMx) as the sediment classification placed it as circalittoral mixed sediment habitat (SS.SMx), *Cerianthus lloydii* was observed and Modiolus was absent.

LL7 PSD would define it as circalittoral fine mud (SS.SMU.CFiMu), with the high abundance of *Amphiura chiajei* suggesting either "*Brissopsis lyrifera* and *Amphiura chiajei* in circalittoral mud" (SS.SMu.CFiMu.BlyrAchi) or "Atrina fragilis and echinoderms on circalittoral mud" (SS.SMu.CFiMu.AtrEch). However, there are several key species absent for both of these habitat types and a high abundance of *Scalibregma inflatum*, *Abra nitida* and *Nucula* species, which are more indicative of "*Abra alba* and *Nucula nitidosa* in circalittoral muddy sand or slightly mixed sediment" (SS.SSa.CMuSa.AalbNuc).

LL11 PSD would define it as circalittoral fine mud circalittoral fine mud (SS.SMU.CFiMu), with the high abundance of *Amphiura filiformis* suggesting "*Atrina fragilis* and echinoderms on circalittoral mud" (SS.SMu.CFiMu.AtrEch). However, as with station LL7, there are several key species (*Cyclista lacerate*, *Atrina fragilis* and *Alcyonium digitatum*) absent and a high abundance of *Abra alba*, *Scalibregma inflatum* and *Nucula* species, which are more indicative of "*Abra alba* and *Nucula nitidosa* in circalittoral muddy sand or slightly mixed sediment" (SS.SSa.CMuSa.AalbNuc).

LL13 was defined as "*Kurtiella bidentata* and *Thyasira* spp. in circalittoral muddy mixed sediment" (SS.SMx.CMx.KurThyMx) as the sediment classification placed it as circalittoral mixed sediment habitat (SS.SMx) and it had abundant *Thyasira flexuosa*, *Scalibregma inflatum* and *Kurtiella bidentata* and well as many of the other taxa important for defining this habitat type.

LL14 PSD would define it as circalittoral fine mud (SS.SMu.CFiMu), with the high abundance of *Amphiura filiformis* and some *Amphiura chiajei* suggesting "*Atrina fragilis* and echinoderms on circalittoral mud" (SS.SMu.CFiMu.AtrEch). However, as with station LL7, there are several key species (*Cyclista lacerate*, *Atrina fragilis* and *Alcyonium digitatum*) absent and a high abundance of *Abra alba*, which is more indicative of "*Abra alba* and *Nucula nitidosa* in circalittoral muddy sand or slightly mixed sediment" (SS.SSa.CMuSa.AalbNuc).

LL16 PSD would define it as circalittoral fine mud (SS.SMu.CFiMu), but as the sample is dominated by *Abra alba*, "*Abra alba* and *Nucula nitidosa* in circalittoral muddy sand or slightly mixed sediment" (SS.SSa.CMuSa.AalbNuc).

LL17 has a high abundance of *Abra alba*, which isn't a commonly found species for circalittoral mixed sediment habitat (SS.SMx) category. "*Abra alba* and *Nucula nitidosa* in circalittoral muddy sand or slightly mixed sediment" (SS.SSa.CMuSa.AalbNuc) is therefore far more likely. Whilst this does not strictly follow the Particle Size Analysis (PSA) data, the substratum is described as being "Fine muddy sands occasionally with small gravel content". As it is only just above the threshold for being described as a mixed sediment (6.8% gravel), this classification is appropriate.

LL25 Particle Size Distribution (PSD) defines it as circalittoral sandy mud (SS.SMU.CSaMu) with a high abundance of Ophiuridae. However, it contains several habitat characterising species for "Sparse *Modiolus modiolus*, dense *Cerianthus lloydii* and burrowing holothurians on sheltered circalittoral stones and mixed sediment" (SS.SMx.CMx.ClloModHo), namely *Modiolus modiolus*, *Ceriathus lloydii* and a burrowing holothurian (*Leptosynapta*). As this habitat category is mixed sediment typically with a low proportion of gravel (6.72%), this is not far from the 2.45% gravel recorded.

LL26 was defined as "*Kurtiella bidentata* and *Thyasira* spp. in circalittoral muddy mixed sediment" (SS.SMx.CMx.KurThyMx) as the sediment classification placed it as circalittoral mixed sediment habitat (SS.SMx) and it had a high abundance of *Thyasira flexuosa*, *Scalibregma inflatum* and *Kurtiella bidentata* and well as other taxa important for defining this habitat type.

There was no PSD data recorded for LL27 due to the high quantity of stones and shells, suggesting a mixed, coarse or hard substrate. There was a high abundance of *Spirobranchus triqueter* (recorded as *Pomatoceros triqueter*), *Ophiothrix fragilis* and *Ophiocomina nigra*. This suggests that the habitat can be classified as "*Ophiothrix fragilis* and/or *Ophiocomina nigra* brittlestar beds on sublittoral mixed sediment" (SS.SMx.CMx.OphMx), although *Modiolus modiolus* were also identified from this sample.

LL28 is defined by the PSD as a circalittoral sandy mud (SS.SMU.CSaMu), with an even abundance of the species defining "*Amphiura filiformis*, *Kurtiella bidentata* and *Abra nitida* in circalittoral sandy mud" (SS.SMu.CSaMu.AfilKurAnit).

LL30 is defined by the PSD as a circalittoral fine mud (SS.SMu.CFiMu), but is dominated by *Abra nitida*, *Nucula nitidosa* and *Abra alba* with no *Kurtiella bidentata*. We would therefore say that it is most similar to the habitat category "*Abra alba* and *Nucula nitidosa* in circalittoral muddy sand or slightly mixed sediment" (SS.SSa.CMuSa.AalbNuc), which is associated with slightly coarser sediments.

LL32 had some of the component species of "*Abra alba* and *Nucula nitidosa* in circalittoral muddy sand or slightly mixed sediment" (SS.SSa.CMuSa.AalbNuc) and "*Kurtiella bidentata* and *Thyasira* spp. in circalittoral muddy mixed sediment" (SS.SMx.CMx.KurThyMx). The PSD placed

it as circalittoral mixed sediment habitat (SS.SMx), hence it will be defined as "*Kurtiella bidentata* and *Thyasira* spp. in circalittoral muddy mixed sediment" (SS.SMx.CMx.KurThyMx) in spite of the absence of the second to fourth most important taxa (*Thyasira flexuosa*, *Kurtiella bidentata* and *Hilbigneris gracilis*).

LL33 PSD defines it as circalittoral fine mud (SS.SMU.CFiMu), albeit with very similar percentages of sand and mud. The biological community is characteristic of "*Abra alba* and *Nucula nitidosa* in circalittoral muddy sand or slightly mixed sediment" (SS.SSa.CMuSa.AalbNuc), with a high abundance of *Abra alba* and *Nucula nitidosa*.

LL36 was dominated by Mya arenaria and the invasive polychaete *Pseudopolydora paucibranchiata*. However, these taxa which are not included in any of the possible biotopes based on the level 5 EUNIS classification. The most similar biotope is "*Kurtiella bidentata* and *Thyasira* spp. in circalittoral muddy mixed sediment" (SS.SMx.CMx.KurThyMx) as the sediment classification placed it as circalittoral mixed sediment habitat (SS.SMx) and it had a high abundance of *Thyasira flexuosa*, *Scalibregma inflatum* and *Kurtiella bidentata* and well as other taxa important for defining this habitat type.

LL43 PSD defines it as circalittoral fine mud (SS.SMU.CFiMu), with some of the component species of "*Abra alba* and *Nucula nitidosa* in circalittoral muddy sand or slightly mixed sediment" (SS.SSa.CMuSa.AalbNuc) and "*Kurtiella bidentata* and *Thyasira* spp. in circalittoral muddy mixed sediment" (SS.SMx.CMx.KurThyMx). Due to the PSD and the sample being dominated by *Abra alba*, it is defined as (SS.SSa.CMuSa.AalbNuc).

LL46 PSD defines it as circalittoral fine mud (SS.SMU.CFiMu) and it has a biological community is characteristic of "*Abra alba* and *Nucula nitidosa* in circalittoral muddy sand or slightly mixed sediment" (SS.SSa.CMuSa.AalbNuc), with a high abundance of *Abra alba* and *Nucula nitidosa*. The high abundance of *Chaetozone zetlandica* is unusual as it is generally associated with coarser substrates.

LL50 PSD defines it as circalittoral fine mud (SS.SMU.CFiMu) and it has a biological community is characteristic of "*Abra alba* and *Nucula nitidosa* in circalittoral muddy sand or slightly mixed sediment" (SS.SSa.CMuSa.AalbNuc), with a high abundance of *Abra alba* and presence of *Nucula nitidosa*. This also matches the 1.92% gravel content of the sediment, suggesting slightly mixed sediment.

LL54 was defined as "*Kurtiella bidentata* and *Thyasira* spp. in circalittoral muddy mixed sediment" (SS.SMx.CMx.KurThyMx) as the sediment classification placed it as circalittoral mixed sediment habitat (SS.SMx). It had *Disporella hispida* and other epifauna on the larger components of the sediment as well as soft sediment species such as *Thyasira flexuosa*, *Scalibregma inflatum* and *Owenia fusiformis*. However, *Kurtiella bidentata* and Nemertea were absent from the sample, so this is a suggested biotope.

LL65 PSD defines it as circalittoral fine mud (SS.SMU.CFiMu), but has an unusual biological community dominated by Chaetozone species, which are more characteristic of mixed or offshore sediments. No level 5 classification has been suggested.

9 CPET Species

Table 11: An initial investigation into the CPET taxa that are known to be detectable using the Freshwater Invertebrate approach taken in this project. While the known value is that c. 47% of taxa used for the Macroinvertebrates CPET are detectable, it is likely that in reality this proportion is far higher, as there are likely to be many cases where the taxonomy used by the Macroinvertebrates CPET approach is different to that used by NatureMetrics (gbif backbone).

Macroinvertebrates_CPET Species list	Detected in this project	Known from other NatureMetrics projects
Ablabesmyia longistyla	Yes	Yes
Ablabesmyia monilis	Yes	Yes
Ablabesmyia phatta		
Acamptocladius (GENUS)		Yes
Acricotopus lucens		Yes
Anatopynia plumipes		
Apsectrotanypus trifascipennis	Yes	Yes
Arctopelopia (GENUS)	Yes	Yes
Boreoheptagyia (GENUS)		
Brillia flavifrons		
Brillia bifida	Yes	
Bryophaenocladius (GENUS)	Yes	Yes
Camptocladius stercorarius		Yes
Cardiocladius (GENUS)	Yes	Yes
Chaetocladius (GENUS)	Yes	Yes
Chironomus (Lobochironomus) carbonaria		
Chironomus (Lobochironomus) dissidens		
Chironomus annularius		Yes
Chironomus anthracinus		Yes
Chironomus aprilinus		
Chironomus bernensis		
Chironomus cingulatus	Yes	Yes
Chironomus commutatus		Yes
Chironomus dorsalis		
Chironomus holomelas		
Chironomus longipes		
Chironomus longistylus		
Chironomus luridus		Yes
Chironomus macani	Yes	Yes
Chironomus nuditarsis		Yes
Chironomus nudiventris		
Chironomus obtusidens	Yes	Yes
Chironomus pallidivittatus		
Chironomus piger		
Chironomus plumosus group		Yes
Chironomus prasinus		
Chironomus pseudothummi		Yes
Chironomus riparius		Yes
Chironomus salinarius		
Chironomus tentans		Yes
Chironomus (GENUS) OTHER	Yes	Yes

Cladopelma (GENUS)	Yes	Yes
Cladotanytarsus atridorsum	Yes	Yes
Cladotanytarsus difficilis		Yes
Cladotanytarsus lepidocalcar		
Cladotanytarsus new pupal species		Unsure
Cladotanytarsus (GENUS) OTHER	Yes	Yes
Cladotanytarsus vanderwulpi		
Clinotanypus nervosus	Yes	Yes
Clunio marinus		
Conchapelopia melanops	Yes	Yes
Conchapelopia (GENUS) OTHER		Yes
Constempellina brevicosta		
Corynocera ambigua		
Corynoneura arctica group		Unsure
Corynoneura fittkaui		Yes
Corynoneura scutellata group		Yes
Corynoneurella paludosa		
Cricotopus (Cricotopus) bicinctus	Yes	Yes
Cricotopus (Cricotopus) trifascia		Yes
Cricotopus (SUB GENUS Cricotopus) OTHER	Yes	Yes
Cricotopus (Isocladius) brevipalpis		
Cricotopus (Isocladius) intersectus group	Yes	Unsure
Cricotopus (Isocladius) sylvestris (Fabricius group)		Yes
Cricotopus (Isocladius) Pe		Unsure
Cricotopus (SUB GENUS Isocladius) OTHER	Yes	Yes
Crictopus (Nostococladius) lygropis		
Cryptochironomus obreptans group		Unsure
Cryptochironomus redekei group	Yes	Yes
Cryptotendipes (GENUS)		
Demeijerea rufipes	Yes	Yes
Demicryptochironomus (GENUS)	Yes	Yes
Diamesa (GENUS)	Yes	Yes
Dicrotendipes nervosus		Yes
Dicrotendipes notatus		
Dicrotendipes pallidicornis		
Dicrotendipes tritomus	Yes	Yes
Dicrotendipes (GENUS) OTHER	Yes	Yes
Diplocladius cultriger		
Einfeldia pagana		
Endochironomus albipennis		
Endochironomus tendens		Yes
Endochironomus (GENUS) OTHER	Yes	Yes
Epoicocladius ephemerae		
Eukiefferiella ancyla		
Eukiefferiella claripennis	Yes	Yes
Eukiefferiella coerulescens		
Eukiefferiella (GENUS) OTHER	Yes	Yes
Eurycnemus crassipes		
Euryhapsis fusciropes		

Fleuria lacustris		
Georthocladius luteicornis		
Glyptotendipes (SUB GENUS Caulochironomus)		Unsure
Glyptotendipes (SUB GENUS Glycotendipes)		Yes
Glyptotendipes (Trichotanypus) signatus		
Graceus ambiguus		
Guttipelopia guttipennis		
Gymnometriocnemus (GENUS)		Yes
Halocladius (SUB GENUS Halocladius)		
Halocladius (Psammocladius) braunsi		
Harnischia (GENUS)	Yes	Yes
Hayesomyia tripunctata		
Heleniella ornaticollis		
Heterotanytarsus apicalis	Yes	Yes
Heterotrissocladius (GENUS)	Yes	Yes
Kiefferulus tendipediformis	Yes	Yes
Kloosia pusilla		
Krenopelopia (GENUS)	Yes	Yes
Krenosmittia (GENUS)		
Labrundinia longipalpis		
Larsia (GENUS)		Yes
Lauterborniella agrayloides		
Limnophyes (GENUS)	Yes	Yes
Lipiniella araenicola		
Macropelopia adaucta		
Macropelopia nebulosa	Yes	Yes
Macropelopia (GENUS) OTHER		Yes
Metriocnemus (GENUS)	Yes	Yes
Microchironomus tener		Yes
Microchironomus (GENUS) OTHER		yes
Micropsectra atrofasciata	Yes	Yes
Micropsectra fusca		
Micropsectra junci	Yes	Yes
Micropsectra (GENUS) OTHER	Yes	Yes
Microtendipes britteni		
Microtendipes (GENUS) OTHER	Yes	Yes
Monodiamesa bathyphila		
Monodiamesa ekmani		
Monopelopia tenuicalcar		Yes
Nanocladius balticus		
Nanocladius dichromis group	Yes	
Nanacladius rectinervis group	Yes	
Nanocladius (GENUS) OTHER	Yes	Yes
Natarsia (GENUS)		
Neozavrelia longappendiculata		
Neozavrelia (GENUS) OTHER		
Nilotanypus dubius	Yes	Yes
Nilothauma brayi		
Odontomesa fulva		

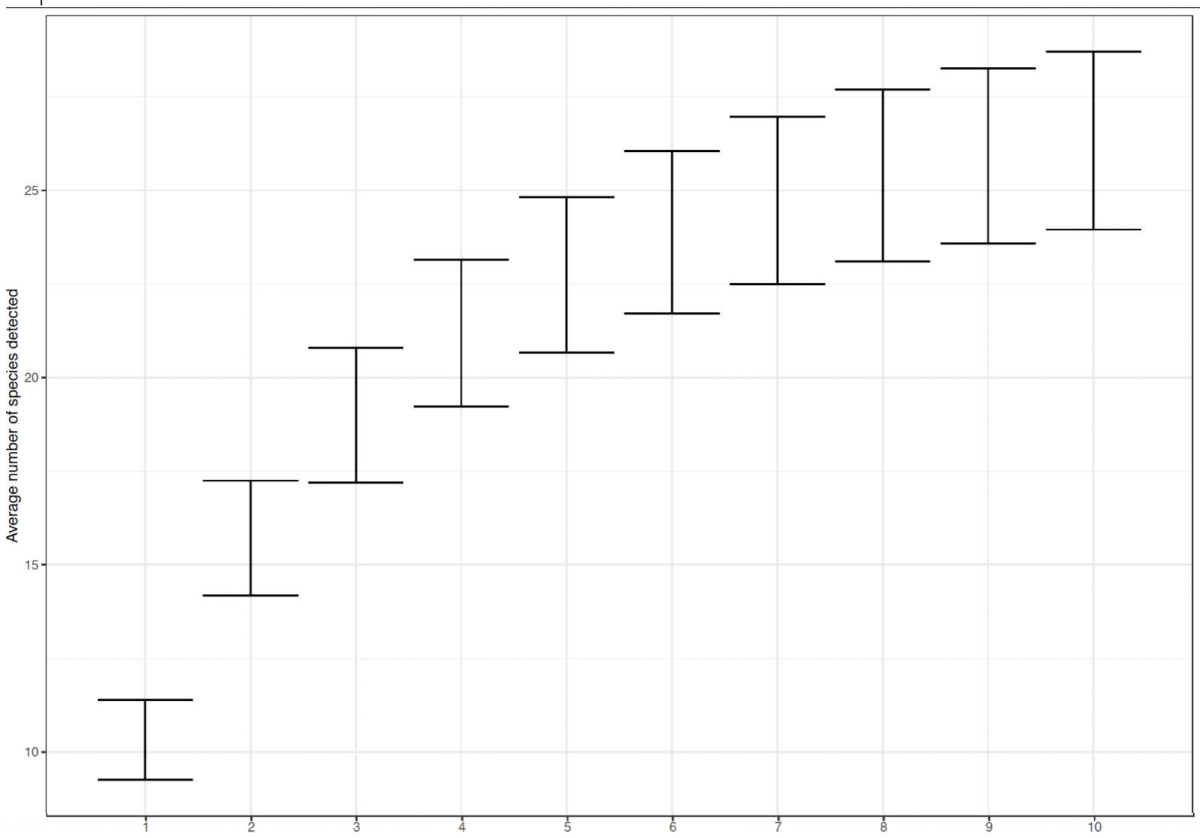
Omisus caledonicus		
Orthocladius (SUB GENUS Eudactylocladius)	Yes	Unsure
Orthocladius (SUB GENUS Euorthocladius)		Unsure
Orthocladius (Orthocladius) frigidus	Yes	Yes
Orthocladius (Orthocladius) rubicundus		Yes
Orthocladius (SUB GENUS Orthocladius) OTHER		Yes
Orthocladius (Pogonocladius) consobrinus	Yes	
Orthocladius (Symposiocladius) holsatus		
Orthocladius (Symposiocladius) lignicola		
Pagastiella orophila		
Parachironomus arcuatus	Yes	Yes
Parachironomus biannulatus	Yes	Yes
Parachironomus frequens	Yes	Yes
Parachironomus tenuicaudatus	Yes	Yes
Parachironomus (GENUS) OTHER	Yes	Yes
Paracladius conversus		
Paracladopelma camptolabis group		
Paracladopelma nigritulum		
Paracricotopus niger		
Parakiefferiella coronata	Yes	Yes
Parakiefferiella fennica		
Parakiefferiella Pe 1		Unsure
Parakiefferiella (GENUS) OTHER	Yes	Yes
Paralauterborniella nigrohalteralis		Yes
Paralimnophyes hydrophilus		Yes
Paramerina (GENUS)		
Parametriocnemus (GENUS)	Yes	Yes
Parorthocladius nudipennis		
Paraphaenocladius (GENUS)	Yes	Yes
Parapsectra nana		
Parapsectra (GENUS) OTHER		
Paratanytarsus laccophilus		
Paratanytarsus tenellulus		Yes
Paratanytarsus (GENUS) OTHER	Yes	Yes
Paratendipes (GENUS)	Yes	Yes
Paratrichocladius rufiventris	Yes	Yes
Paratrichocladius skirwithensis		
Paratrissocladius excerptus		
Paratrichocladius GENUS (OTHER)		Yes
Phaenopsectra (GENUS)	Yes	Yes
Polypedilum (Polypedilum) arundinetum		
Polypedilum (Polypedilum) cultellatum		
Polypedilum (Polypedilum) nubeculosum group		Yes
Polypedilum (Polypedilum) pedestre	Yes	Yes
Polypedilum (Pentapedilum) nubens		
Polypedilum (Pentapedilum) sordens group		Unsure
Polypedilum (Tripodura) pullum group		Unsure
Polypedilum (Tripodura) tetracrenatum		

Polypedilum (GENUS) OTHER	Yes	Yes
Potthastia gaedii group	Yes	Unsure
Potthastia longimana group	Yes	Unsure
Procladius (Holotanypus) crassinervis		
Procladius (SUB GENUS Holotanypus) OTHER	Yes	Unsure
Procladius (SUB GENUS Psilotanypus)	Yes	Unsure
Prodiamesa olivacea	Yes	Yes
Protanypus morio		
Psectrocladius (Psectrocladius) barbimanus		Yes
Psectrocladius (Psectrocladius) brehmi		
Psectrocladius (Psectrocladius) octomaculatus		Yes
Psectrocladius (Psectrocladius) oligosetus		
Psectrocladius (Psectrocladius) schlienzi		
Psectrocladius (Psectrocladius) species A		Unsure
Psectrocladius (SUB GENUS Psectrocladius) OTHER	Yes	Unsure
Psectrocladius (Allopsectrocladius) platypus	Yes	Yes
Psectrocladius (Allopsectrocladius) obvius		
Psectrocladius (Mesopsectrocladius) barbatipes		
Psectrocladius (Monopsectrocladius) calcaratus	Yes	
Psectrotanypus varius	Yes	Yes
Pseudochironomus prasinatus	Yes	Yes
Pseudodiamesa (GENUS)		
Pseudokiefferiella parva		
Pseudorthocladius (GENUS)	Yes	Yes
Pseudosmittia (GENUS)	Yes	Yes
Rheocricotopus (SUB GENUS Psilocricotopus)		Unsure
Rheocricotopus (SUB GENUS Rheocrictopus)	Yes	Unsure
Rheopelopia (GENUS)		Yes
Rheosmittia spinicornis		
Rheotanytarsus (GENUS)	Yes	Yes
Saetheria reissi		
Schineriella schineri		
Sergentia (GENUS)	Yes	
Smittia (GENUS)	Yes	Yes
Stempellina almi		
Stempellina bausei	Yes	Yes
Stempellinella (GENUS)	Yes	Yes
Stenochironomus (GENUS)		
Stictochironomus (GENUS)	Yes	Yes
Sympotthastia zavreli		
Syndiamesa edwardsi		
Synendotendipes (GENUS)		
Synorthocladius semivirens	Yes	Yes
Tanypus punctipennis		Yes
Tanypus (GENUS) OTHER		Yes
Tanytarsus anderseni		
Tanytarsus brundini	Yes	Yes

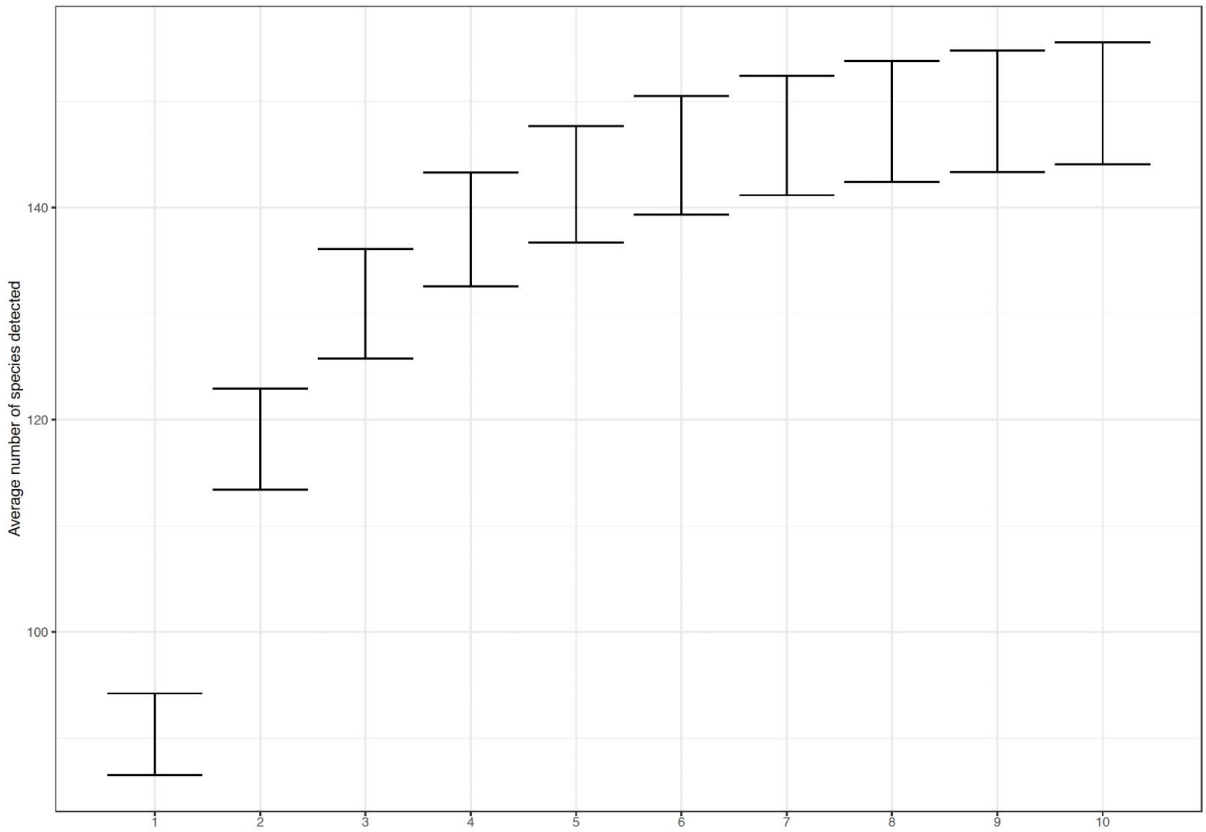
Tanytarsus buchonius		Yes
Tanytarsus chinyensis		
Tanytarsus ejuncidus group		Yes
Tanytarsus gracilentis		
Tanytarsus mendax	Yes	Yes
Tanytarsus pallidicornis		Yes
Tanytarsus signatus	Yes	Yes
Tanytarsus striatulus	Yes	Yes
Tanytarsus sylvaticus	Yes	Yes
Tanytarsus (SUB GENUS Part 1) OTHER	Yes	Unsure
Tanytarsus (SUB GENUS Part 2) OTHER	Yes	Unsure
Tanytarsus (SUB GENUS Part 3) OTHER		Unsure
Telmatopelopia nemorum		
Telopelopia (GENUS)		
Thalassosmittia thalassophilus		
Thienemannia (GENUS)		Yes
Thienemanniella (GENUS)	Yes	Yes
Thienemannimyia (GENUS)	Yes	Yes
Tokunagaia tonolli		
Tribelos intextus	Yes	Yes
Trissocladius brevipalpis		
Trissopelopia longimana	Yes	Yes
Tvetenia discoloripes		
Tvetenia (GENUS) OTHER	Yes	Yes
Virgatanytarsus (GENUS)	Yes	Yes
Xenochironomus xenolabis		Yes
Xenopelopia (GENUS)		Yes
Zalutschia humphresiae		
Zavrelia pentatoma		
Zavreliella marmorata		
Zavrelimyia nubila		
Zavrelimyia (GENUS) OTHER	Yes	Yes

10 Sample Replicate Plots – Marine

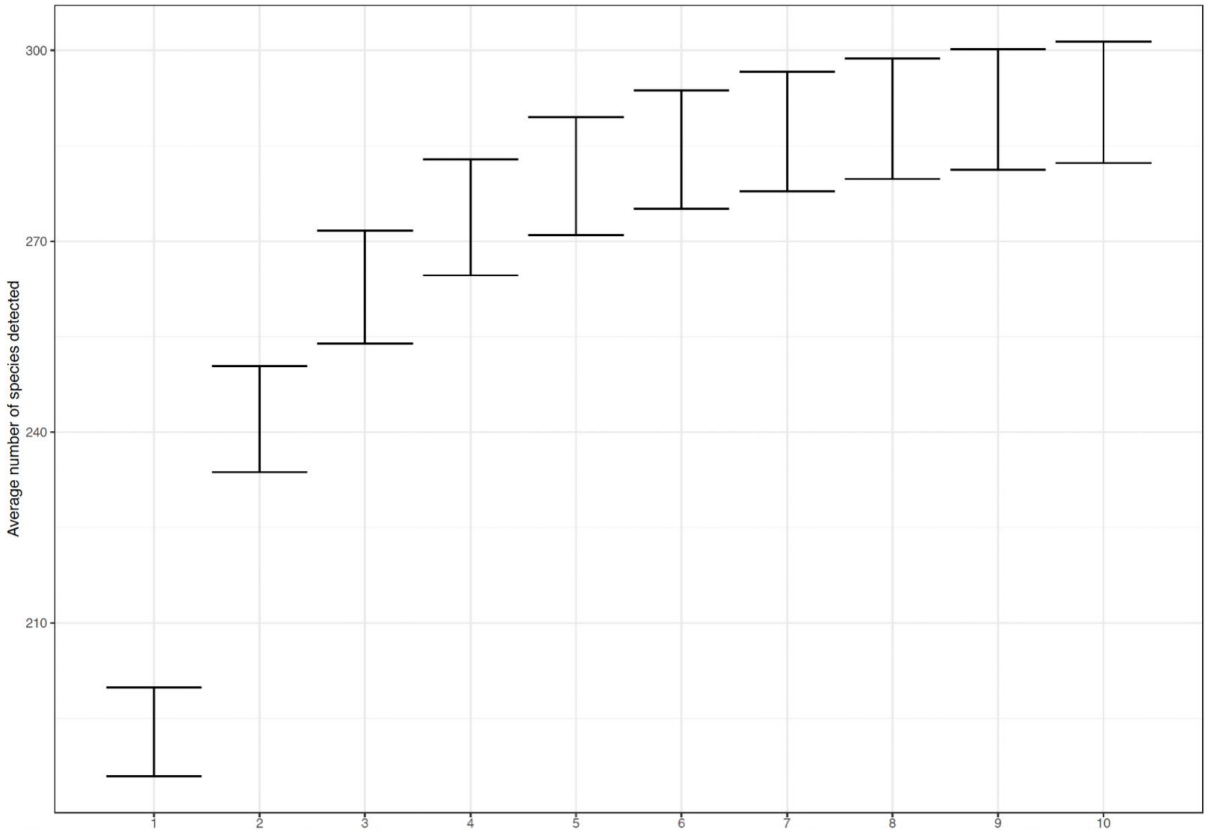
For each species, the m=3 sample replicates per station allow us to use occupancy modelling to estimate probability distributions of detection **theta** (q) and of occupancy **psi** (y), both of which normally have mean values less than 1. We can combine q and y to calculate the probability of detecting a species at a typical station: $\psi(1-(1-\theta)^m)$ where m is the number of sample replicates. For instance, if q=0.47 and y=0.5 for species 1, the detection probability with m=5 sample replicates is 48%: $0.5(1-(1-0.47)^5)=0.479$); we can think of species 1 as contributing just under half a species to the station's expected detected species richness. We calculated these values for all species, over each species' occupancy and detection probability distributions, for 1 to 10 sample replicates, and summed over all species to estimate a range of species detected for each value of m.



Marine sediment invertebrates



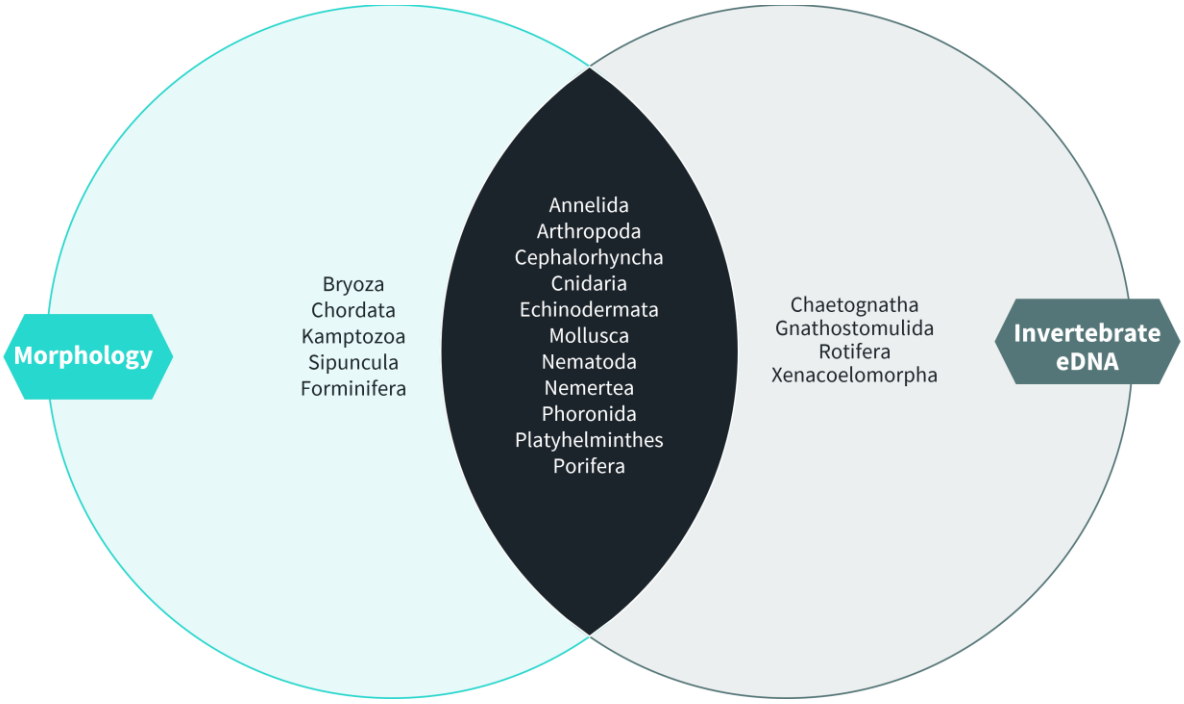
Marine sediment eukaryotes

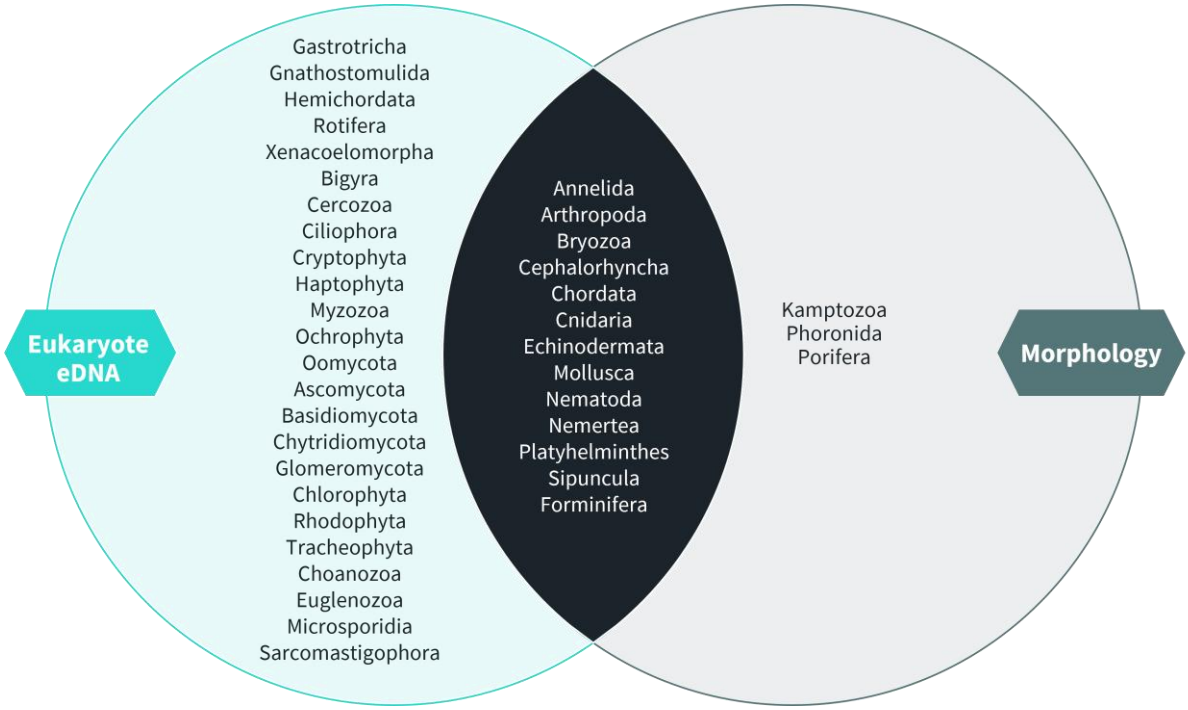


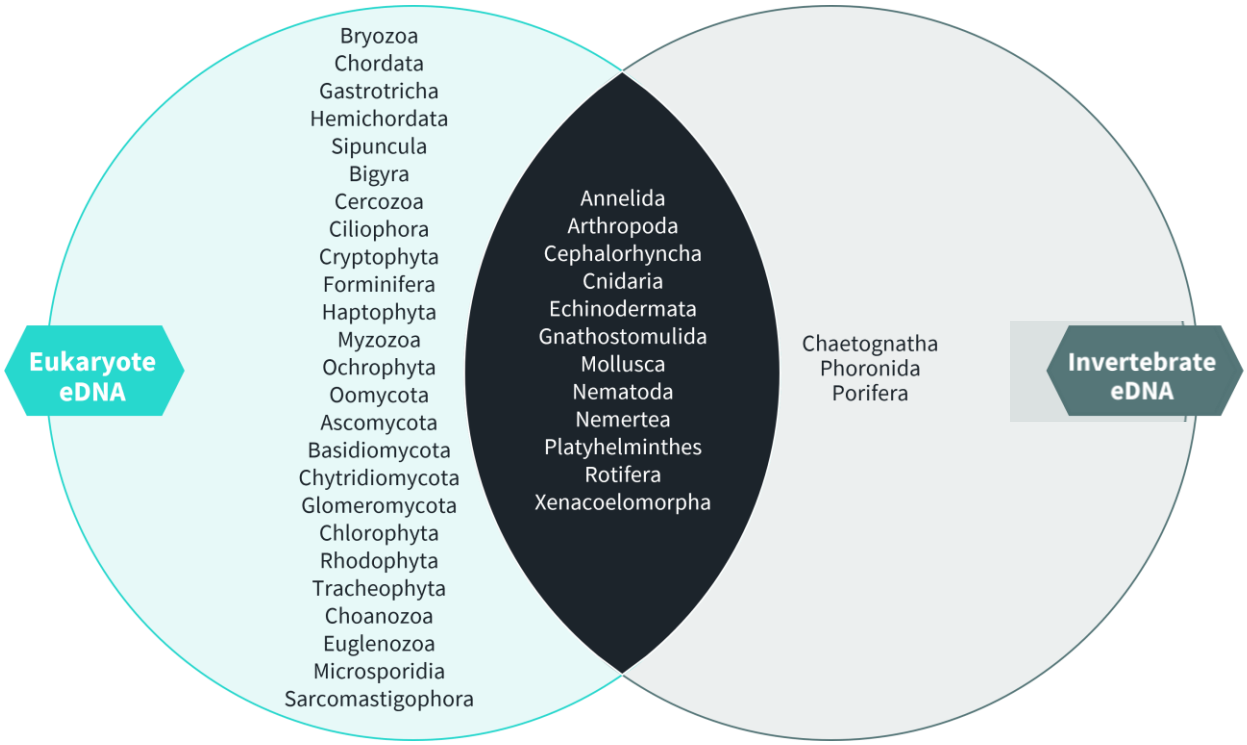
Marine sediment bacteria

11 Morphology vs eDNA – Marine Invertebrates & Eukaryotes

Venn diagrams showing the phylum level detection overlap between visual morphology and eDNA-based metabarcoding. Between morphology and Invertebrate (18S) eDNA metabarcoding, between morphology and Eukaryote (COI) eDNA metabarcoding, and between Invertebrate (18S) and Eukaryote (COI) eDNA metabarcoding.




Eukaryote eDNA
Gastrotricha
Gnathostomulida
Hemichordata
Rotifera
Xenacoelomorpha
Bigyra
Cercozoa
Ciliophora
Cryptophyta
Haptophyta
Myzozoa
Ochrophyta
Oomycota
Ascomycota
Basidiomycota
Chytridiomycota
Glomeromycota
Chlorophyta
Rhodophyta
Tracheophyta
Choanozoa
Euglenozoa
Microsporidia
Sarcomastigophora
Annelida
Arthropoda
Bryozoa
Cephalorhyncha
Chordata
Cnidaria
Echinodermata
Mollusca
Nematoda
Nemertea
Platyhelminthes
Sipuncula
Forminifera
Kamptozoa
Phoronida
Porifera
Morphology


Eukaryote eDNA
Bryozoa
Chordata
Gastrotricha
Hemichordata
Sipuncula
Bigyra
Cercozoa
Ciliophora
Cryptophyta
Forminifera
Haptophyta
Myzozoa
Ochrophyta
Oomycota
Ascomycota
Basidiomycota
Chytridiomycota
Glomeromycota
Chlorophyta
Rhodophyta
Tracheophyta
Choanozoa
Euglenozoa
Microsporidia
Sarcomastigophora
Annelida
Arthropoda
Cephalorhyncha
Cnidaria
Echinodermata
Gnathostomulida
Mollusca
Nematoda
Nemertea
Platyhelminthes
Rotifera
Xenacoelomorpha
Chaetognatha
Phoronida
Porifera
Invertebrate eDNA

12 Historical Fish Records - Freshwater

Historical records of fish for each of the freshwater lochs included in the project were compiled from the following sources:

Adams. C.E., The fish community of Loch lomond, Scotland: its history and rapidly changing status. Hydrobiologia 290: 91-102, 1994
Etheridge, E.C. & Adams. C., Bream (Abramis brama), a new fish species confirmed in Loch Lomond. Glasgow Naturalist, 2008, 25, 93-94
Grant, A, Duguid, A & Adams, C. Reappearance of tench (Tinca tinca L.) in the waters of Loch Lomond. Glasgow Naturalist, 1997, 59-60.
Adams, C.E, Brown, D, & Tippet, R. 1990. Dace & Chub: new introductions to the Loch Lomond catchment. Glasgow Naturalist, 21, 509-513.
SEPA Loch Field Survey Records, Loch Lubnaig, 2010
NBN Atlas - UK's largest collection of biodiversity information
Fish Lochaber | Loch & River Arkaig
Scottish Flyfisher - Argyle & Bute
St.Winnoch Angling Club Home Page (lochwinnochac.net)
Fishing | Local activities | Sunart Adventures
Fish Lochaber | Strontian
Loch Voil and Doine Permits - Angling Active Blog - Fishing News, Advice and Articles
Home - Lake of Menteith Fisheries (menteith-fisheries.co.uk)
Loch Chon | Fishing In The Trossachs
Loch Lomond Angling Improvement Association – Managed by anglers, for anglers (Pers. Comms. G. Bourhill (2023)

This is not considered to be a fully comprehensive list, but represents the best available matching data for the project.

Table 12. Historical Records of fish presence in freshwater lochs. "Y" denotes that one or more records were found for the presence of the respective fish species in the respective loch.

Species	Castle Semple Loch	Lake of Menteith	Loch Achray	Loch Ard	Loch Arkaig	Loch Avich	Loch Chon	Loch Doilet	Loch Eilt	Loch Lomond (North)	Loch Lomond (South)	Loch Lubnaig	Loch Scammadale	Loch Tulla	Loch Voil
Abramis brama											Y				
Anguilla anguilla	Y		Y	Y		Y	Y		Y	Y	Y	Y	Y	Y	Y
Carassius carassius										Y	Y				
Coregonus lavaretus										Y	Y				
Chelon labrosus											Y				
Esox lucius	Y	Y	Y	Y	Y	Y	Y			Y	Y	Y	Y	Y	
Gasterosteus aculeatus	Y		Y			Y			Y	Y	Y	Y			Y
Gobio gobio										Y	Y				
Gobiusculus flavescens													Y		
Gymnocephalus cernua										Y	Y				
Lampetra fluviatilis	Y		Y							Y	Y				
Lampetra planeri	Y		Y			Y				Y	Y				
Leuciscus cephalus										Y	Y				
Leuciscus leuciscus										Y	Y				
Barbatula barbatula	Y		Y							Y	Y				
Oncorhynchus mykiss	Y	Y	Y			Y				Y	Y	Y	Y	Y	

Species	Castle Semple Loch	Lake of Menteith	Loch Achray	Loch Ard	Loch Arkaig	Loch Avich	Loch Chon	Loch Doilet	Loch Eilt	Loch Lomond (North)	Loch Lomond (South)	Loch Lubnaig	Loch Scammadale	Loch Tulla	Loch Voil
Perca fluviatilis	Y		Y			Y	Y			Y	Y	Y		Y	
Petromyzon marinus										Y	Y				
Phoxinus phoxinus	Y		Y	Y			Y		Y	Y	Y	Y			Y
Platichthys flesus			Y							Y	Y			Y	
Pomatoschistus minutus													Y		
Pungitius pungitus										Y	Y				
Rutilus rutilus	Y		Y			Y				Y	Y				
Salmo ferox					Y										
Salmo salar	Y		Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Salmo trutta	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Salmo trutta fario	Y		Y	Y		Y	Y			Y	Y	Y		Y	
Salmo trutta trutta	Y		Y			Y				Y	Y				
Salvelinus alpinus			Y	Y		Y	Y		Y	Y	Y	Y			Y
Tinca tinca										Y	Y				
Trisopterus minutus													Y		
Barbus barbus										Y	Y				

13 AMBI Results

Table 13: Binary (presence vs no detection) was ultimately used for AZTI Marine Biotic Index (AMBI) and genetic AMBI (gAMBI) scoring. Results for each sample for the binary scoring and final condition are presented here.

Sample	AMBI	gAMBI	AMBI Condition	gAMBI condition
LL03.1		2.25	NA	Good
LL03.2		2.333	NA	Good
LL03.3	1.544	2.5	Good	Good
LL03.Mean		2.361		Good
LL07.1		1.5	NA	Good
LL07.2		3.75	NA	Moderate
LL07.3	2.15	2.25	Good	Good
LL07.Mean		2.5		Good
LL11.1		2.5	NA	Good
LL11.2		3.6	NA	Moderate
LL11.3	2.55	2.5	Good	Good
LL11.Mean		2.867		Good
LL13.1		2.464	NA	Good
LL13.2		3	NA	Good
LL13.3	2.313	3.214	Good	Good
LL13.Mean		2.893		Good
LL14.1		2.25	NA	Good
LL14.2		5.25	NA	Poor
LL14.3	2.167	4.5	Good	Poor
LL14.Mean		4		Poor
LL16.1		3.9	NA	Moderate
LL16.2		3.375	NA	Moderate
LL16.3	3	3.9	Good	Moderate
LL16.Mean		3.725		Moderate
LL17.1		0.75	NA	High
LL17.2		3.333	NA	Moderate
LL17.3	2.375	1.875	Good	Good
LL17.Mean		1.986		Good
LL25.1		3.75	NA	Moderate
LL25.2		2.25	NA	Good
LL25.3	1.72	1.75	Good	Good
LL25.Mean		2.583		Good
LL26.1		2.75	NA	Good
LL26.2		2.625	NA	Good
LL26.3	1.99	2.25	Good	Good
LL26.Mean		2.542		Good
LL28.1		2	NA	Good
LL28.2		3.25	NA	Good
LL28.3	1.806	2.25	Good	Good
LL28.Mean		2.5		Good
LL30.1		3	NA	Good
LL30.2		1.5	NA	Good

LL30.3	2.1	1.5	Good	Good
LL30.Mean		2		Good
LL32.1		2.25	NA	Good
LL32.2		3	NA	Good
LL32.3	2.4	7	Good	Bad
LL32.Mean		4.083		Bad
LL33.1		3	NA	Good
LL33.2		7	NA	Bad
LL33.3	2.25	3	Good	Good
LL33.Mean		4.333		Good
LL36.1		1.5	NA	Good
LL36.2		3	NA	Good
LL36.3	1.723	2.455	Good	Good
LL36.Mean		2.318		Good
LL43.1		3	NA	Good
LL43.2		2	NA	Good
LL43.3	2.51	2.063	Good	Good
LL43.Mean		2.354		Good
LL46.1		2.625	NA	Good
LL46.2		4.5	NA	Poor
LL46.3	2.464	2.25	Good	Good
LL46.Mean		3.125		Good
LL50.1		7	NA	Bad
LL50.2		1.5	NA	Good
LL50.3	2.313	0	Good	High
LL50.Mean		2.833		Good
LL54.1		3	NA	Good
LL54.2		2	NA	Good
LL54.3	1.541		Good	NA
LL54.Mean		2.5		Good
LL65.1		3.75	NA	Moderate
LL65.2		4.5	NA	Poor
LL65.3	2.543	3	Good	Good
LL65.Mean		3.75		Moderate

14 Gapfinder Outputs

See separate Excel spreadsheet provided.

15 OTU Tables

These are provided in individual spreadsheets for each habitat type, with different taxon groups presented on different tabs.

16 Site Photographs

This section provides example site photographs for the Woodland and Peatland habitats sampled during the Phase 2 eDNA survey to provide context on the variability of sites Condition categories. Not all sites are included. All photographs were taken by NatureMetrics during field sampling.

16.1 Woodland

16.1.1 Unforested

Coille Coire Chuilc, Loch Lomond and the Trossachs National Park (LLTNP) (Image credit: Hayley Craig)

Coille Ruigh, Glen Affric (Image credit: Marco Fioratti)

Dundreggan Allt Fearna, Glen Moriston (Image credit: Hayley Craig)

Ghubnais, Glen Affric (Image credit: Hayley Craig)

Glen Falloch, LLTNP (Image credit: Hayley Craig)

Rothiemurchus estate, Cairngorms National Park (CNP) (Image credit: Hayley Craig)

16.1.2 Recently Planted/Regenerating

Coille Ruigh, Glen Affric (Image credit: Marco Fioratti)

Dundreggan Allt Fearna, Glen Moriston (Image credit: Marco Fioratti)

Ghubnais, Glen Affric (Image credit: Hayley Craig)

Glen Falloch, LLTNP (Image credit: Hayley Craig)

Glen More, CNP (Image credit: Marco Fioratti)

Rothiemurchus estate, CNP (Image credit: Hayley Craig)

16.1.3 Mature

Coille Coire Chuilc, LLTNP (Image credit: Hayley Craig)

Coille Ruigh, Glen Affric (Image credit: Marco Fioratti)

Ghubnais, Glen Affric (Image credit: Hayley Craig)

Glen Falloch, LLTNP (Image credit: Hayley Craig)

Glen More, CNP (Image credit: Marco Fioratti)

Rothiemurchus estate, CNP (Image credit: Hayley Craig)

16.2 Peatland

16.2.1 Degraded

Auchlyne, LLTNP (Image credit: Hannah Flintham)

Auchlyne, LLTNP (Image credit: Hannah Flintham)

Cashel, LLTNP (Image credit: Hayley Craig)

Glen Finglas, LLTNP (Image credit: Hayley Craig)

16.2.2 Restored

Auchlyne, LLTNP (Image credit: Hannah Flintham)

Auchlyne, LLTNP (Image credit: Hannah Flintham)

Glen Finglas, LLTNP (Image credit: Hayley Craig)

Glen Finglas, LLTNP (Image credit: Hayley Craig)

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