Supplementary Documents

S1. Summary of Applied Genomics Methods

The Supplementary Document summarises methods from Applied Genomics (2023) which explains the methods used for wider sample collection, processing and sequencing. Any text taken directly from the report is presented in quotation marks.

Sample Site Collection:

Applied Genomics supplied ezDNA aquatic eDNA sampling kits and ecoType landscape eDNA sampling kits which were used in all cases. All samples were collected by The Bug Farm from 31st July 2023 to 22nd August 2023.

“The Applied Genomics ezDNA sampling kits have been specifically designed for simple and cost-effective water sampling of environmental DNA by non-specialist citizen scientists. The ezDNA sampler is a small, field-portable manual pump device which enables filtration of up to 5 litres of water through a small pore filter capsule with prefilter. Using a small pore size filter (0.45um) maximises the probability of eDNA capture whilst the integrated prefilter facilitates filtration of particle-laden waters. The ezDNA pump attaches to the outflow of the filter, reducing sample cross-contamination risks and the amount of single-use plastics used in the sampling process.

The ecoType eDNA sampling kits enables collection of up to 200g surface sediment per sample, which would be collected as a subsample from benthic physicochemistry sample grabs. The larger sample volume improves probability of detection and reduces noise between replicates. This technique enables direct correlation of targeted benthic invertebrate communities with environmental pressures.

For sample quality assurance, Applied Genomics provided a non-toxic, non-hazardous DNA preservative for stabilisation and transport of all samples. We have included a synthetic DNA internal positive control, with additional field and laboratory negative controls to ensure DNA integrity and reliability of results.”

Sample Processing and Sequencing:

Below is an outline of the full sample processing that occurred, from Applied Genomics.

“Sample processing took place in a dedicated laboratory, separate from downstream processes, to minimize the risk of sample contamination. Each filter capsule sample was homogenized using a benchtop vortex mixer. The liquid contents were transferred to centrifuge tubes and organic matter was concentrated through high-speed centrifugation. DNA purification was performed using QIAGEN DNeasy Blood & Tissue kits, and DNA quantification was done with the Qubit 3.0 High Sensitivity kit following the manufacturer's instructions. A negative extraction blank was included in the purification step as a control.

For PCR amplification, specific DNA primers targeting marine eukaryote "barcode" regions (MiFish for the mitochondrial 12S ribosomal subunit and CytB for the mitochondrial Cytochrome B) were used to cover a wide range of detectable fish communities. PCR replicates (minimum of 3) were

pooled to increase the detection probability. Amplicons were purified using AMPure XP magnetic beads and quality checked with capillary electrophoresis on a QIAGEN QIAxcel Advanced instrument using a DNA Screening kit.

To prepare amplicon sequencing libraries, a Nextera XTv2 index kit and Illumina's methods were employed. Library purification was carried out using AMPure XP beads, and quality was checked using capillary electrophoresis. Library concentrations were quantified, adjusted, pooled, denatured with NaOH, and supplemented with PhiX control following Illumina's protocols. The eDNA amplicon library was sequenced on an Illumina MiSeq sequencer using 2x300bp V3 chemistry."

Following this, a bioinformatic pipeline was applied, resulting in taxonomic assignment (Figure 1).

"To ensure comparability of detected sequences across studies, we utilized the DADA2 algorithm for creating exact amplicon sequence variants (ASVs). This approach models and corrects sequenced amplicon errors, providing a significant advantage over operational taxonomic units (OTUs) by enabling direct comparison of ASVs between studies. OTU methods group similar sequences based on an arbitrary similarity threshold, while DADA2 infers the underlying sample sequences by modelling amplicon errors, leading to improved specificity and sensitivity in identifying true sequence variance and rare variants."

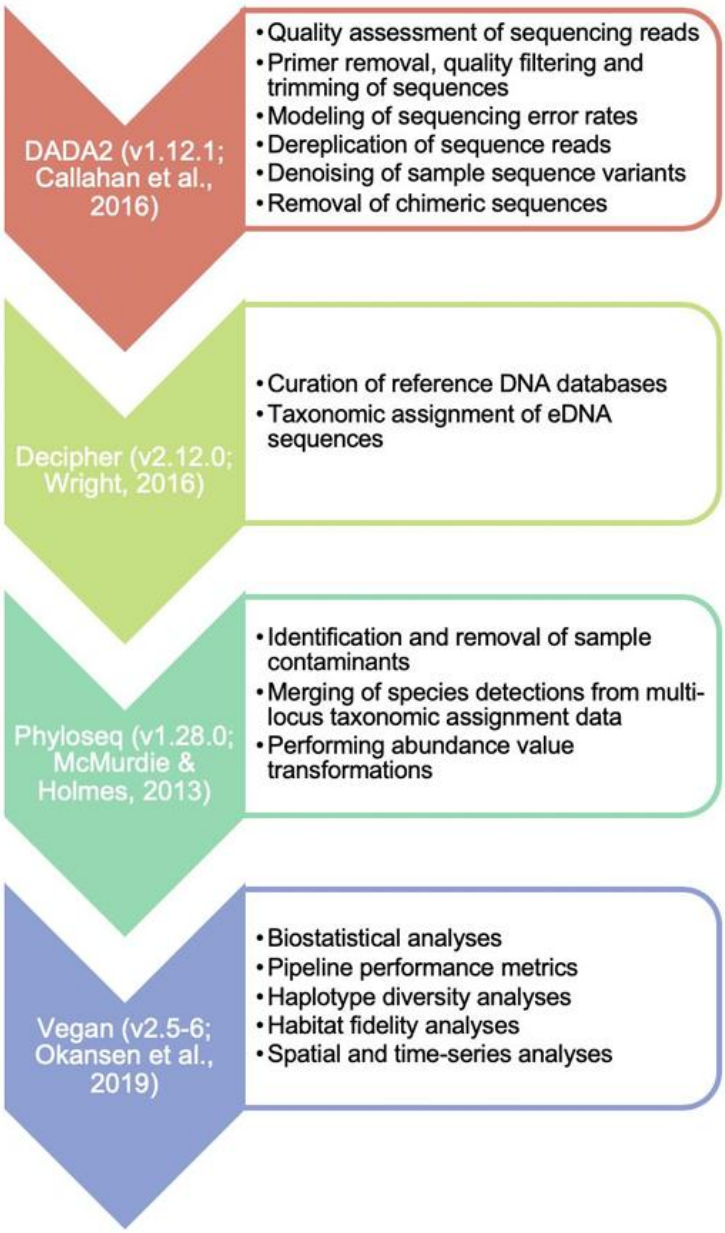

Figure 1: Outline of bioinformatics and taxonomic assignment methodology, produced by Applied Genomics.

To ensure the quality of the data, the performance of the bioinformatics and statistical analyses were assessed as follows.

“The confidence scores for taxonomic assignment of each ASV were visualized, showing the range based on the fraction of bootstrap replicates assigned to the species rank. The accuracy of taxonomic assignments relied on the quality of our curated reference databases and the specificity of barcode loci for discriminating closely related species.

To evaluate the risk of false detections or misclassification errors, we conducted false positive detection analyses. This involved a naive Bayesian approach, considering uncertainties in taxonomic assignments and incorporating historical data of prior detections for each species.

Threshold indicator taxa were identified to assess gradients of environmental conditions using taxon-specific change-point identification. Nonmetric multidimensional scaling (NMDS) was

employed to visualize dissimilarities between invertebrate community groupings. Beta diversities of inshore benthic communities were evaluated using permutational multivariate analyses of variance (PERMAVOVA).

To ensure credibility and reproducibility, we considered results statistically significant at $p < 0.05$, following convention, and intrinsically credible at $p < 0.001$."

S2: Supplementary Analyses

Additional statistics:

Table 1: Pairwise Wilcoxon signed rank tests for observed diversity

	Intensive Grassland	Marshy Grassland	Pond	River
Marshy Grassland	0.42844248	NA	NA	NA
Pond	0.020957486	0.000128669	NA	NA
River	0.082909985	0.008653369	0.991249	NA
Wildflower Meadow	1	0.392683665	0.000129	0.008653

Table 2: Pairwise Wilcoxon signed rank tests for Shannon diversity

	Intensive Grassland	Marshy Grassland	Pond	River
Marshy Grassland	0.428442	NA	NA	NA
Pond	0.020957	0.000129	NA	NA
River	0.08291	0.008653	0.991249	NA
Wildflower Meadow	1	0.392684	0.000129	0.008653

Table 3: Betadisper results for Habitat, Conditions, and Station

(a) Betadisper ANOVA for Habitat

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	1	0.034299	0.034299	10.54432	0.002205
Residuals	45	0.146379	0.003253	NA	NA

(b) Betadisper ANOVA for Conditions

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	4	0.115891	0.028973	8.507301	4.02E-05
Residuals	42	0.143037	0.003406	NA	NA

(c) Betadisper ANOVA for Station

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Groups	2	0.103848	0.051924	8.44579	0.000787
Residuals	44	0.270509	0.006148	NA	NA

Table 4: Indicator species associated with Conditions (IG = Intensive Grassland, MG = Marshy Grassland, WM = Wildflower Meadow)

Species	**IG**	**MG**	**WM**	**Phylum**	**Trophic Mode**	**IndVal stat**	**P value**
Syncephalis sp.	1	0	0	Zoopagomycota	Pathotroph	0.74535599	0.01
Pseudoramichloridium henryi	0	1	0	Ascomycota	Pathotroph-Saprotroph	0.74095857	0.045
Humicola nigrescens	1	0	0	Ascomycota	Pathotroph-Saprotroph	0.93541435	0.004
Achroceratosphaeria sp.	0	1	0	Ascomycota	Saprotroph	0.68313005	0.018
Schizothecium curvuloides	1	0	1	Ascomycota	Saprotroph	0.72872886	0.05
Brocchiosphaera brocchiata	0	1	0	Ascomycota	Unknown	0.73029674	0.015
Tolypocladium cylindrosporum	1	1	0	Ascomycota	Pathotroph-Symbiotroph	0.82213296	0.047
Beauveria sp.	1	0	0	Ascomycota	Pathotroph-Saprotroph-Symbiotroph	0.77598359	0.024
Emericellopsis glabra	1	0	0	Ascomycota	Saprotroph	0.96609178	0.002
Clonostachys candelabrum	0	1	0	Ascomycota	Pathotroph-Saprotroph	0.68581427	0.03
Gloniacea (gen) *Incertae sedis* sp.	0	0	1	Ascomycota	Unknown	0.66421116	0.019
Geomyces auratus	0	1	0	Ascomycota	Saprotroph	0.68313005	0.012
Exophiala radicis	1	0	0	Ascomycota	Pathotroph-Saprotroph-Symbiotroph	0.81779570	0.021
Dictyosporium elegans	1	0	0	Ascomycota	Saprotroph	0.74095857	0.034
Torula gaodangensis	1	0	0	Ascomycota	Saprotroph-Symbiotroph	1	0.001
Tympanidacea (gen) *Incertae sedis* sp.	0	1	0	Ascomycota	Unknown	0.81649658	0.026
GS29 (gen) *Incertae sedis* sp.	0	1	0	Basidiomycota	Unknown	0.68313005	0.011
Panaeolina foenisecii	1	0	1	Basidiomycota	Saprotroph	0.74376408	0.029
Clavaria sp.	0	1	1	Basidiomycota	Saprotroph-Symbiotroph	0.90971765	0.017
Marasmius nigrobrunneus	1	0	0	Basidiomycota	Pathotroph-Saprotroph-Symbiotroph	0.77598359	0.018
Filobasidiella sp.	1	0	0	Basidiomycota	Pathotroph-Saprotroph	0.81649658	0.006
Mortierella sp.	1	0	0	Mortierellomycota	Saprotroph-Symbiotroph	0.85990501	0.014

Podila humilis	1	1	0	Mortierellomycota	Saprotroph-Symbiotroph	0.78173596	0.045
Hormiactis sp.	1	0	0	Ascomycota	Pathotroph	0.81649658	0.006
Pseudogymnoascus sp.	1	0	1	Ascomycota	Pathotroph-Saprotroph-Symbiotroph	0.88546805	0.001
Pseudogymnoascus sp.	1	0	0	Ascomycota	Pathotroph-Saprotroph-Symbiotroph	0.80260313	0.022
Neoascochyta paspali	1	1	0	Ascomycota	Pathotroph-Saprotroph	0.70710678	0.023
Lulwoana sp.	0	1	0	Ascomycota	Saprotroph	0.85634884	0.001
Dendrophoma sp.	0	0	1	Ascomycota	Pathotroph	0.53452248	0.046
Fusarium nisikadoi	0	1	0	Ascomycota	Pathotroph-Saprotroph-Symbiotroph	0.68313005	0.015
Slooffia sp.	1	0	0	Basidiomycota	Saprotroph	0.77598359	0.016
Linnemannia camargensis	1	1	0	Mortierellomycota	Saprotroph-Symbiotroph	0.89232357	0.001
Acrostalagmus luteoalbus	1	0	0	Ascomycota	Symbiotroph	0.71415560	0.035
Vishniacozyma dimennae	1	0	0	Basidiomycota	Saprotroph	0.77598359	0.02
Trichosporon sp.	1	0	0	Basidiomycota	Pathotroph-Saprotroph	0.81649658	0.006
Preussia flanaganii	1	0	0	Ascomycota	Saprotroph	0.77849894	0.011

Table 5: Indicator species associated with Station (IG = Intensive Grassland, PS =Protected Sites, HC = Habitat Creation)

Species	**IG**	**PS**	**HC**	**Phylum**	**Trophic mode**	**IndVal statistic**	**P value**
Syncephalis sp.	1	0	0	Zoopagomycota	Pathotroph	0.732743	0.036
Humicola nigrescens	1	0	0	Ascomycota	Pathotroph-Saprotroph	0.953463	0.001
Apiospora kogelbergensis	1	0	0	Ascomycota	Pathotroph-Saprotroph-Symbiotroph	0.696311	0.026
Chaetosphaeria sp.	0	1	0	Ascomycota	Saprotroph-Symbiotroph	0.661396	0.047
Hypomyces gamsii	0	1	0	Ascomycota	Pathotroph	0.761387	0.012
Beauveria sp.	1	0	0	Ascomycota	Pathotroph-Saprotroph-Symbiotroph	0.787499	0.011
Emericellopsis glabra	1	0	0	Ascomycota	Saprotroph	0.9759	0.001
Phialocephala bamuru	0	1	0	Ascomycota	Saprotroph-Symbiotroph	0.57735	0.041
Orbiliales (gen) *Incertae sedis* sp.	0	1	0	Ascomycota	Unknown	0.57735	0.032
Hyaloscypha gryndleri	0	1	0	Ascomycota	Pathotroph-Saprotroph-Symbiotroph	0.57735	0.042

Exophiala radicis	1	0	0	Ascomycota	Pathotroph-Saprotroph-Symbiotroph	0.84182	0.01
Subulicystidium perlongisporum	0	1	0	Basidiomycota	Saprotroph	0.57735	0.028
Stagonospora sp.	0	1	0	Ascomycota	Pathotroph	0.686156	0.038
Dictyosporium elegans	1	0	0	Ascomycota	Saprotroph	0.761387	0.014
Torula gaodangensis	1	0	0	Ascomycota	Saprotroph-Symbiotroph	1	0.002
Penicillium corylophilum	0	1	0	Ascomycota	Pathotroph-Saprotroph-Symbiotroph	0.686156	0.03
Penicillium burgense	0	1	0	Ascomycota	Pathotroph-Saprotroph-Symbiotroph	0.851835	0.001
Clavaria sp.	0	1	1	Basidiomycota	Saprotroph-Symbiotroph	0.909718	0.013
Marasmius nigrobrunneus	1	0	0	Basidiomycota	Pathotroph-Saprotroph-Symbiotroph	0.787499	0.012
Ceratobasidiacea (gen) *Incertae sedis* sp.	0	1	0	Basidiomycota	Pathotroph-Saprotroph-Symbiotroph	0.761387	0.012
Filobasidiella sp.	1	0	0	Basidiomycota	Pathotroph-Saprotroph	0.816497	0.008
Mortierella sp.	1	0	0	Mortierellomycota	Saprotroph-Symbiotroph	0.873334	0.007
Apenidiella foetida	0	1	0	Ascomycota	Pathotroph-Saprotroph	0.666667	0.033
Hormiactis sp.	1	0	0	Ascomycota	Pathotroph	0.816497	0.008
Pseudogymnoascus sp.	1	0	0	Ascomycota	Pathotroph-Saprotroph-Symbiotroph	0.827291	0.008
Fusarium nisikadoi	0	1	0	Ascomycota	Pathotroph-Saprotroph-Symbiotroph	0.686156	0.033
Slooffia sp.	1	0	0	Basidiomycota	Saprotroph	0.787499	0.014
Acrostalagmus luteoalbus	1	0	0	Ascomycota	Symbiotroph	0.711568	0.044
Vishniacozyma dimennae	1	0	0	Basidiomycota	Saprotroph	0.787499	0.012
Trichosporon sp.	1	0	0	Basidiomycota	Pathotroph-Saprotroph	0.816497	0.008
Preussia flanaganii	1	0	0	Ascomycota	Saprotroph	0.755929	0.02

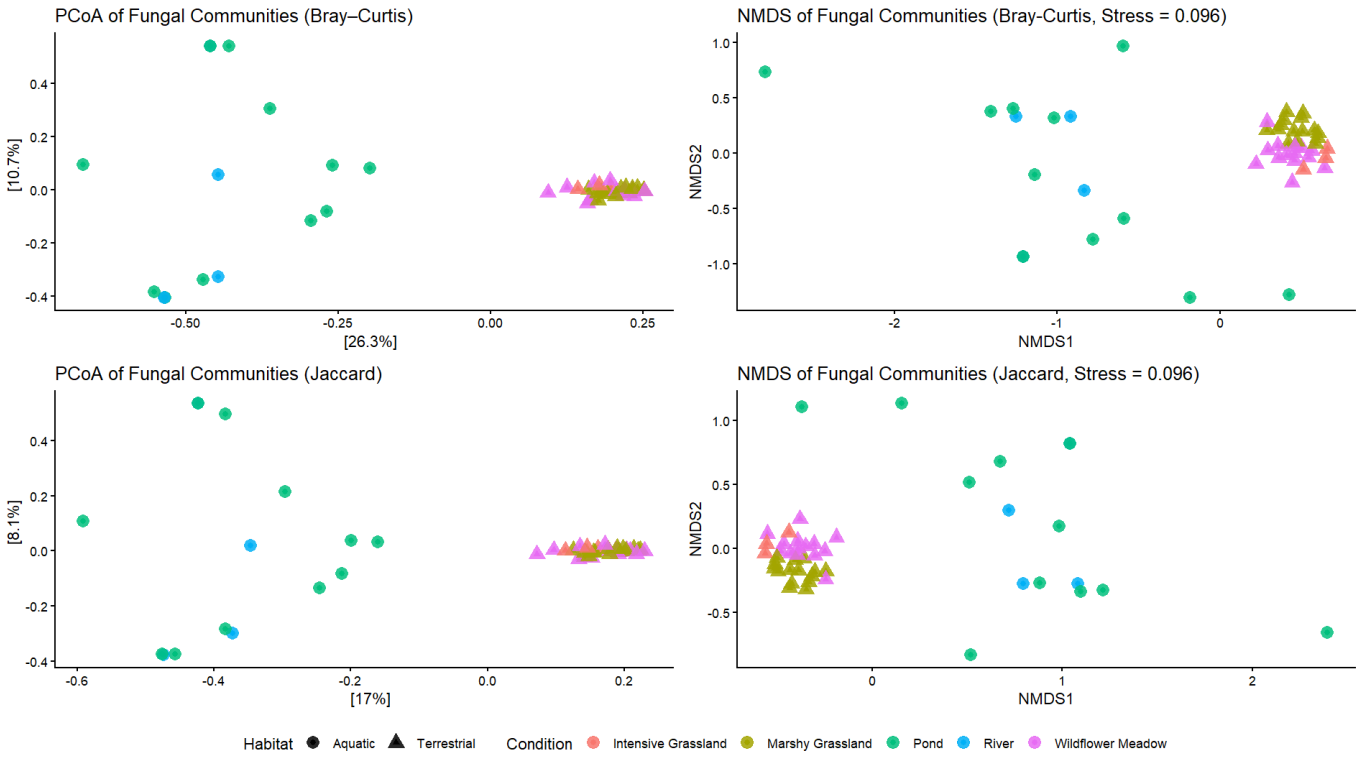

Figure 2: Alternative representation of PCoA (left) and NMDS (right) data using both Bray-Curtis and Jaccard dissimilarity matrices. Conditions are denoted by colour. Each point represents either an aquatic sample site (circular) or a terrestrial sample site (triangular).

Additional analyses to inform habitat management

In addition to the analyses carried out in the study, several site-specific visualisations were conducted in order to inform certain land management practices and can be found below. These included comparisons of Shannon diversity between habitat creation and protected sites for pond samples (Figure 3) and marshy grassland (Figure 4), as these were the only two Conditions that had enough replicates in both protected sites and habitat creation sites for statistical comparison to be viable. The overall difference between habitat creation and protected sites is included in the main report but because these contain a mix of habitats, the differences here could be because the differing Conditions harbour more/less diversity, rather than differences due to land management (Station). As well as this, proportional representations of trophic mode composition for each individual aquatic site were created (Figures 5 and 6).

The occurrence of grassland waxcaps (genus Hygrocybe) was visualised (Figure 7). Interestingly, Hygrocybe sp. were not detected at any intensive grassland sample sites. This aligns broadly with the literature regarding waxcaps, and is likely due to the fertiliser application, ploughing, and grazing that has occurred more frequently on these sites. In contrast, the presence of waxcaps in the marshy grasslands and wildflower meadows highlights their importance in maintaining fungal biodiversity. Because a high proportion of waxcap species appear on European Red Data Lists and some depend on established, oligotrophic grasslands, the conservation and maintenance of habitats such as these is essential for supporting these fungi.

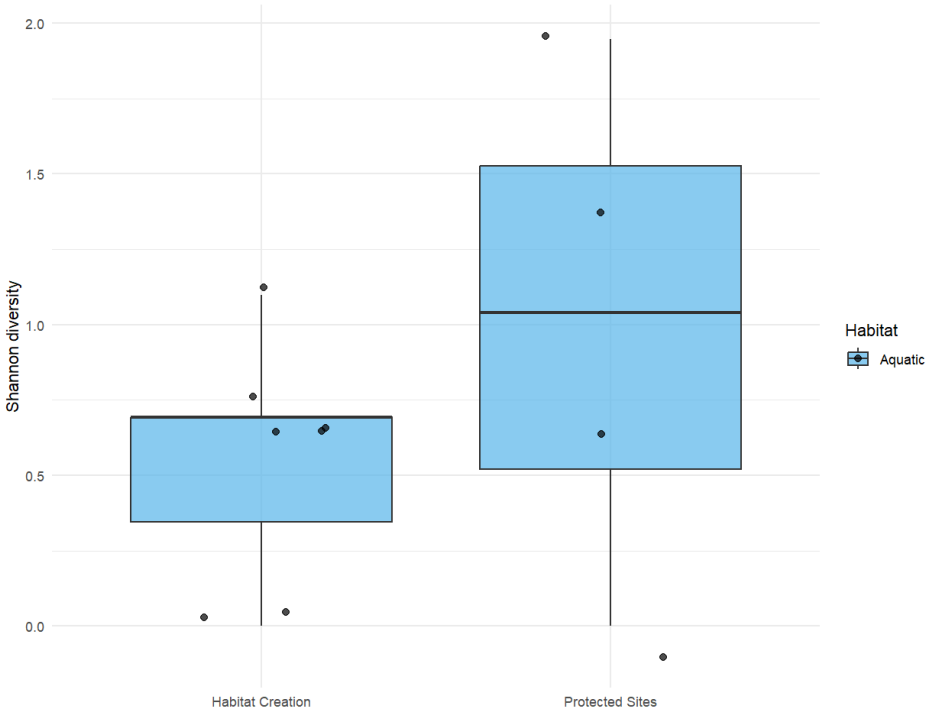

Figure 3: Spread of Shannon diversity between aquatic **pond** samples by habitat creation (n=7) and protected sites (n=4).

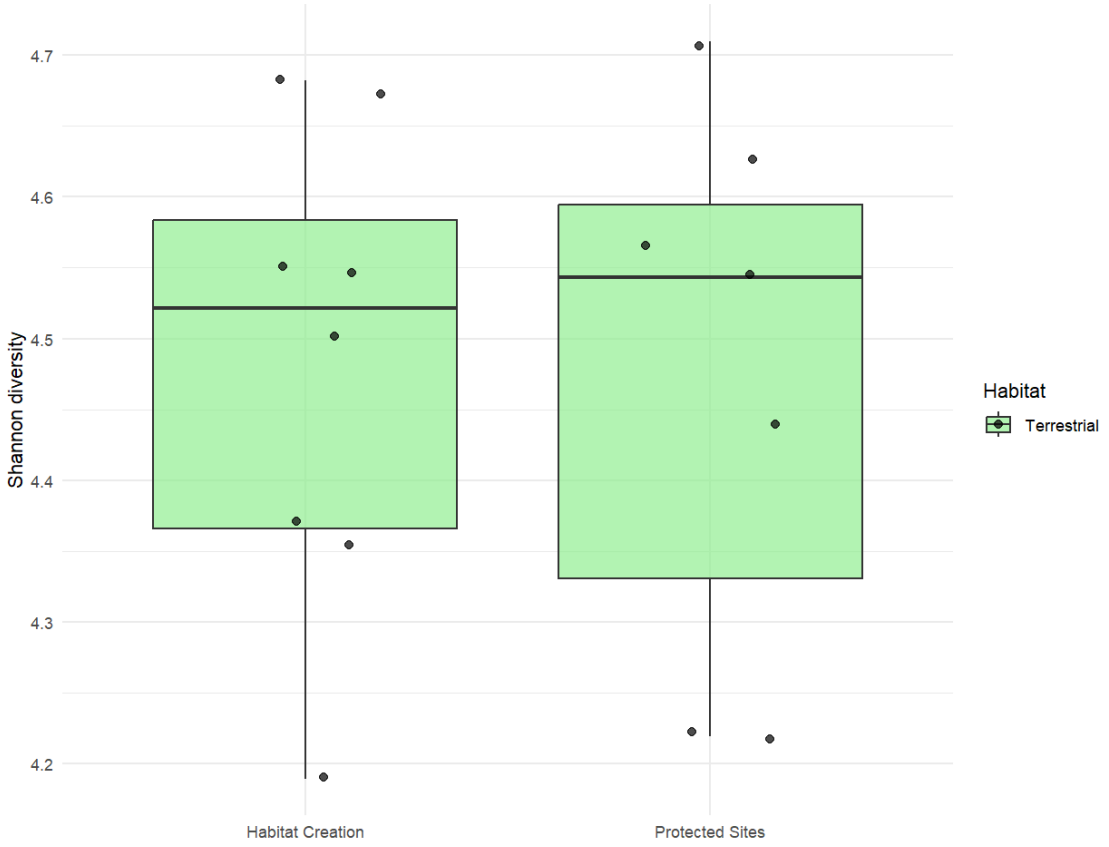

Figure 4: Spread of Shannon diversity between terrestrial **marshy grassland** samples by habitat creation (n=8) and protected sites (n=7).

Wilcoxon rank sum tests found no significant difference between stations for marshy grassland (W = 26.5, p-value = 0.9077) or for pond (W = 9, p-value = 0.3676).

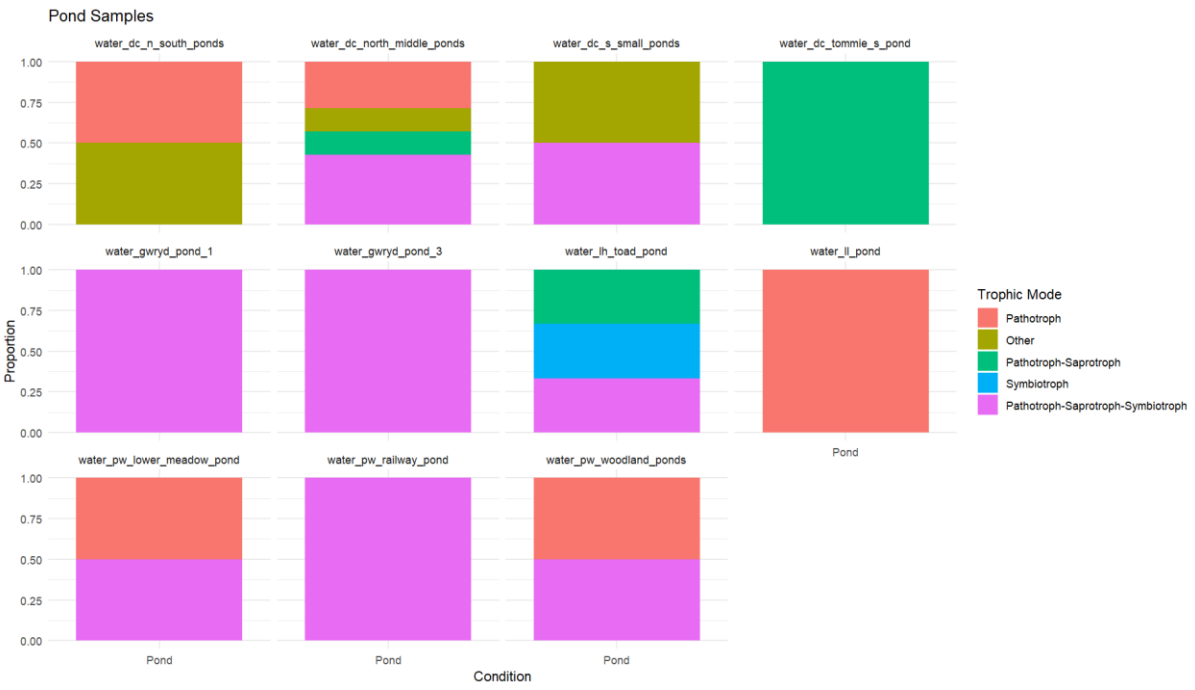

Figure 5: Proportion of Trophic Modes in individual pond samples.

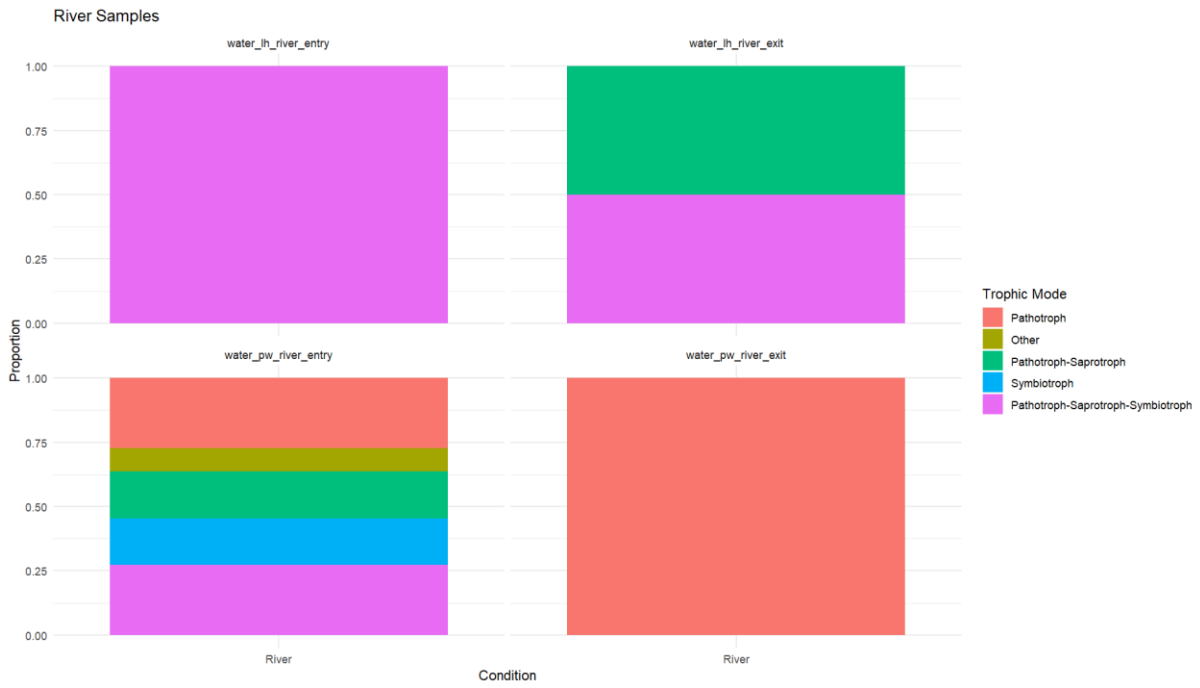

Figure 6: Proportion of Trophic Modes in individual river samples.

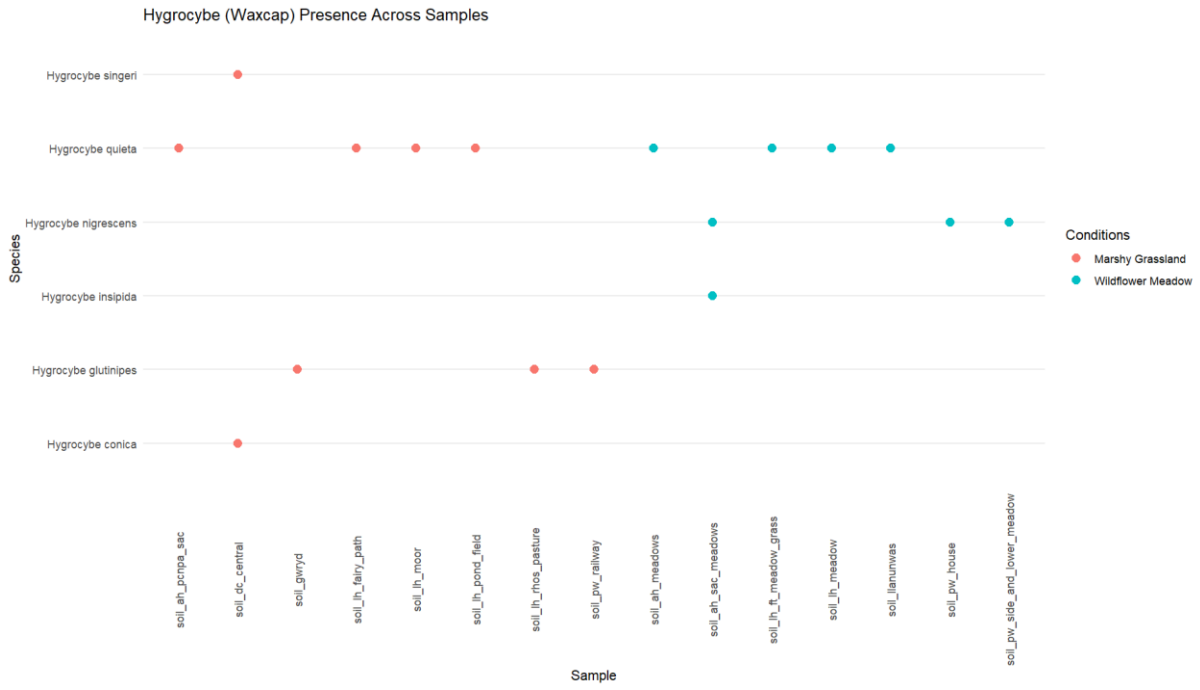

Figure 7: Waxcap species (genus Hygrocybe) detected across sample sites. There were 17 detected occurrences across 15 samples.