Assessing how fungal diversity varies by land use across the St Davids peninsula using eDNA sampling

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1. Abstract

Fungi underpin essential ecosystem processes, yet their diversity and responses to land management practices remain understudied, particularly at a local level. eDNA metabarcoding now offers a more representative way to characterise fungal communities in comparison to traditional surveying. This study used eDNA sampling across 57 sites (32 soil, 25 water) on the St Davids peninsula, Pembrokeshire, encompassing five habitat conditions (wildflower meadow, marshy grassland, intensive grassland, ponds, rivers) and three land use stations (protected sites, intensive grassland, habitat creation). Diversity metrics and ordination techniques were used to assess how habitat and land use shaped fungal diversity and community composition.

Terrestrial habitats supported significantly higher diversity compared to aquatic habitats, though diversity did not differ among condition or station. Community composition differed strongly between aquatic and terrestrial habitats and showed distinct assemblages in wildflower meadows and marshy grasslands. Aquatic samples were dominated by Ascomycota and pathotrophic guilds (indicative of pollution), whereas terrestrial sites exhibited a broader range of trophic modes and phyla. These results highlight the ecological importance of both wildflower meadows and marshy grasslands as hosts of unique fungal communities and indicate potential water quality issues linked to agricultural runoff and contamination from grazing livestock. More broadly, they demonstrate the power of eDNA sampling in revealing below ground biodiversity and support evidence-based land management techniques aimed at nature recovery on a local scale.

2. Introduction

Fungi are essential components of both terrestrial and aquatic ecosystems, driving key processes such as decomposition and nutrient cycling, as well as influencing the establishment and diversity of plants (Yan et al., 2018). Despite this, fungal communities remain understudied, largely because conventional approaches (primarily fruiting body surveys) fail to capture microfungal diversity and depend heavily on expert identification (Frøslev et al., 2019). This has left major gaps in the understanding of fungal diversity in soil and freshwater habitats, where many fungal taxa are microscopic. Advances in environmental DNA (eDNA) metabarcoding have enabled the characterisation of such communities and have become particularly useful in the detection of fungi in soils and aquatic habitats.

Local biodiversity knowledge is important in effective conservation and land management. For areas such as the St Davids peninsula, Pembrokeshire, where protected sites and restoration sites sit alongside intensive agriculture, understanding how below ground biodiversity responds to differing land use is essential in ensuring nature recovery strategies are effective. Fungal communities are highly sensitive to environmental change, responding to shifts in biotic and abiotic factors such as vegetation structure, nutrient composition, and disturbance and can therefore be used as early indicators of ecosystem recovery or degradation (Li and Dong, 2025). Importantly, the ecological roles of fungi can be interpreted through their trophic modes: Saprotrophs decompose organic matter, symbiotrophs form mutualistic associations with host species, and pathotrophs obtain nutrients from other organisms by harming host cells, though some species also occupy differing roles depending on their life stage (Nguyen et al., 2016). Thus, assessing fungal communities can help guide habitat creation efforts and provide a more holistic view of ecosystem health and function.

This study uses eDNA metabarcoding to characterise aquatic and terrestrial fungi across differing habitats on the St Davids peninsula. Specifically, it aims to assess differences in fungal diversity and composition between aquatic and terrestrial habitats, and to establish whether differing habitat conditions result in distinct fungal assemblages. By describing ecological factors effecting fungal biodiversity and detecting taxa and trophic modes associated with particular habitat conditions, this study aims to provide insight into the value of fungi as indicators of ecosystem health, which can be used to inform conservation and restoration strategies across the peninsula.

3. Methods

3.1 Study Area

A total of 57 samples (32 soil, 25 water) were collected from a range of locations across the St Davids peninsula, Pembrokeshire, Wales (Figure 1). These sites incorporated a variety of habitat types including marshy grasslands (n=15), wildflower meadows (n=14), intensive grasslands (n=3), ponds (n=19), and rivers (n=4) which are representative of the dominant natural, semi natural, and farmed habitats found across the peninsula. The sample sites encompassed three differing land management categories:

1. Protected Sites (n=17) which included Sites of Special Scientific Interest (SSSI) and Special Areas of Conservation (SAC) at Dowrog Common SSSI, St Davids Airfield Heaths SSSI, St Davids Airfield SSSI and Waun Fawr SSSI.
2. Habitat Creation (n=36) at Lower Harglodd Farm (The Bug Farm), Penweathers Farm, the Gwryd, and Llanunwas.
3. Intensive grasslands or clover ley (n=3) at Upper Harglodd Farm, Penberi Farm, and Carn-Nwchwn Farm (St Davids Bunkbarns).

Dowrog Common, St Davids Airfield Heaths and Waun Fawr make up part of the Northwest Pembrokeshire Commons SAC. They consist of extensive tracts of wet and dry heaths with pools and fen. St Davids Airfield SSSI is a tract of dry wildflower meadows, cut for hay. Lower Harglodd Farm is a mixed arable and (Welsh Black) cattle farm, farmed for nature, with nature recovery at the heart of all decision-making. The farm is a mosaic of dry hay meadows (at various stages of reversion after either natural regeneration or sowing), arable land, marshy grassland, Rhos pasture, new heath creation, new ponds (dug since 2024) and new woodland (planted in 2023), with a river running through the site. Pesticides and herbicides have not been used on the farm since approximately 2017, with an intensive programme of habitat creation and management since 2020. Penweathers

Farm is comprised of habitats similar to Lower Harglodd Farm and is managed similarly, by the same landowner. However, parts were farmed more intensively until 2020. The Gwryd is an area of marshy grassland and bog that is grazed by ponies, but is adjacent to intensive dairy grazing at Penberi Farm. Penberi Farm is an intensive ryegrass-based dairy farm while Carn-Nwchwn is an intensive largely arable farm, with samples taken from a short-term clover ley that is also used as a campsite. At many sites there are potential nutrient inputs from adjacent land, both into the soil and water bodies.

Sites were chosen as part of a Nature Networks Fund project to generate baseline ecological data across project sites rather than as part of a scientific study design, hence uneven replication across land management categories.

Figure 1: A map of the St Davids peninsula. Location of sample sites are marked as dots, with blue dots representing aquatic sites and orange dots representing terrestrial sites.

3.2 Field Sampling

Samples were collected by Bug Farm personnel as per the methods outlined by Applied Genomics (see supplementary document, section S1). Soil samples were collected using ecoType landscape eDNA sampling kits, and water samples were collected using ezDNA aquatic sampling kits. Both kits were provided by Applied Genomics. One pooled sample was collected per field, protected area management unit, pond or river section.

3.3 Sample Processing and Taxonomic Assignment

All laboratory processing and bioinformatics were conducted by Applied Genomics Ltd. For the full methods used, refer to supplementary document section S1. DNA was extracted and amplified using fungal Internal Transcribed Spacer (ITS) primers consistent with those used by the Earth Microbiome Project. The amplicon sequencing library was purified and sequenced on an Illumina MiSeq sequencer. Sequences were processed into Amplicon Sequence Variants (ASVs) and taxonomy was assigned using several packages in R.

Following taxonomic assignment, sequences matching the same species were considered haplotypic variants, representing intraspecific genetic diversity. The two haplotype datasets (one for soil samples, one for water samples) were then combined into a master document which was used in subsequent analyses.

3.4 Statistics

All analyses of the data and the production of figures was carried out using R v 4.4.2. To reduce bias present in metabarcoding and to account for the fact that read counts are not considered proportional to abundance, the combined haplotype data was converted into presence/absence for community analysis. The dataset was filtered by kingdom to ensure all taxa were fungal. Samples with zero fungal ASVs after filtering (n = 10) as well as aquatic habitat categories represented by fewer than three samples (ditch and scrape, n = 2) were excluded from statistical analyses due to redundancy or insufficient replication.

A phyloseq object was created using the phyloseq package (McMurdie and Holmes, 2013). This grouped the ASV table, a taxonomy table, and sample metadata into one object, enabling analysis and graphical representation of complex phylogenetic data. Site names were used to group samples into 'Habitat' (terrestrial or aquatic), 'Condition' (wildflower meadow, marshy grassland, intensive grassland, pond, river), and 'Station' (protected sites, habitat creation, intensive grassland). For clarity, these terms will be capitalised throughout the document to distinguish them from their non-technical uses.

Alpha diversity was calculated using both observed richness and Shannon diversity metrics using Wilcoxon rank sum test to assess significant differences between Habitat and aquatic Conditions, and Kruskal-Wallis to assess significant differences among terrestrial Conditions.

The vegan package (Oksanen et al., 2025) was used to visualise community composition across Conditions. Principal Coordinates Analysis (PCoA) and Non-metric Multidimensional Scaling (NMDS) ordinations were used. Bray-Curtis and Jaccard dissimilarity matrices were both generated for this purpose. Differences in community composition across Habitat, Condition, and Station were tested using permutation based multivariate analysis (PERMANOVA). This was carried out using the adonis2 function as part of the vegan package, using 999 permutations. To test validity of these results, betadisper tests were conducted for all PERMANOVAs.

Indicator species analysis for both Condition and Station were run using the multipatt function from the indicspecies package (De Cáceres and Legendre, 2009) using 999 permutations. The IndVal statistic was calculated for each taxon based on its specificity (uniqueness) and fidelity (frequency) to a habitat group, identifying taxa that characterise particular land-use types. Significant ASVs were visualised using bubble plots.

Fungal guild and trophic mode were analysed using FUNGuildR (v.0.3.0; https://CRAN.R-project.org/package=FUNGuildR) installed from GitHub, which uses the FUNGuild database (Nguyen et al., 2016). Data inputted included the ASV sequence, taxonomy, and sample sites. The function fungal_assign was used to link original ASV sequences with associated trophic mode and guild. Differences in trophic mode and were visualised using stacked bar plots separated by habitat type for both Condition and Station. Stacked bar plots were also used to visualise differences in phylum composition using the same groupings.

4. Results

4.1 Fungal diversity

Alpha diversity differed significantly between aquatic and terrestrial habitats (Figure 2). Terrestrial samples exhibited significantly higher richness and diversity with both observed richness and Shannon diversity indicating clear differences ($W = 0$, $p < 0.001$ for both metrics).

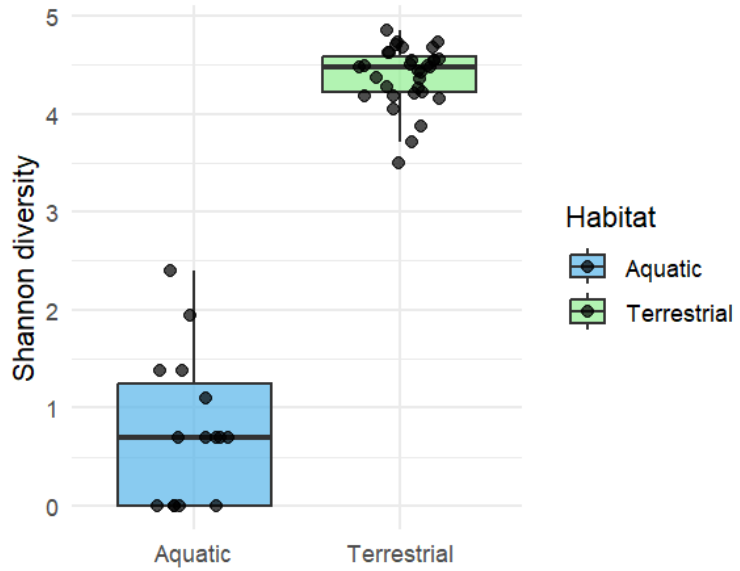

Figure 2: The difference in Shannon diversity between aquatic samples (blue) and terrestrial samples (green). Points represent individual aquatic and terrestrial sample sites.

There was significant difference in alpha diversity across Conditions (Figure 3). Kruskal-Wallis tests found significant variation between different Conditions for both observed richness ($\chi^2 = 30.87$, $df = 4$, $p < 0.001$) and Shannon diversity ($\chi^2 = 30.87$, $df = 4$, $p < 0.001$). Post-hoc analysis using pairwise Wilcoxon signed rank tests (with Benjamini Hochberg correction) indicated that wildflower meadow, marshy grassland, and intensive grassland supported significantly higher fungal diversity than pond and river sites. There were no significant differences in diversity between pond and river, or between wildflower meadow, marshy grassland, and intensive grassland (tables T1 and T2, supplementary document).

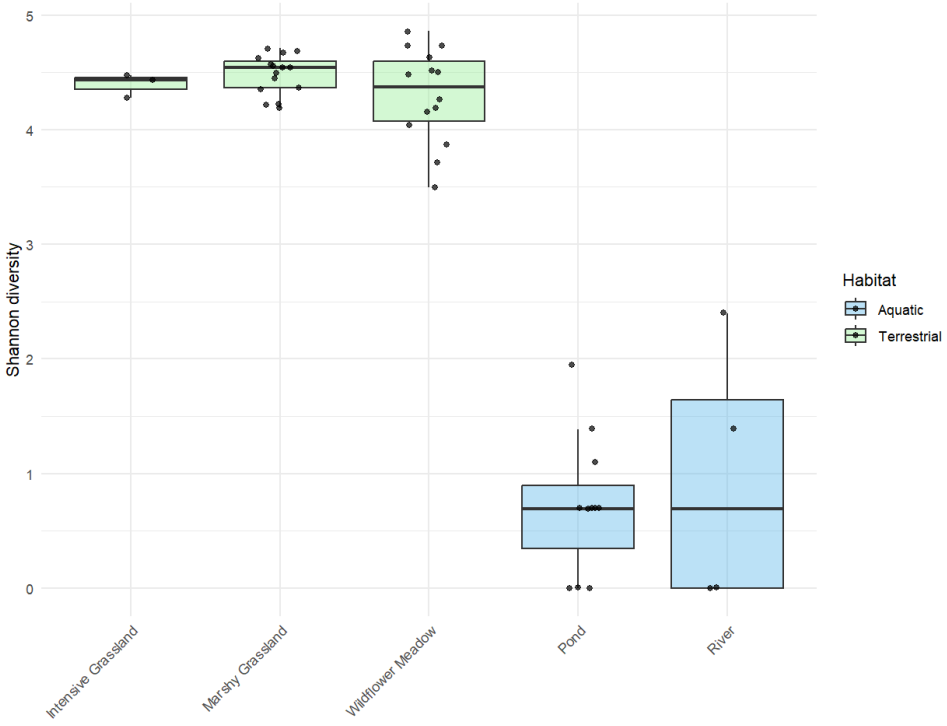

Figure 3: Differences in Shannon diversity between Conditions. Terrestrial Conditions are displayed in green, aquatic Conditions are displayed in blue. Points represent individual sample sites.

No significant differences in observed or Shannon diversity among aquatic or terrestrial stations were detected (Figure 4). Wilcoxon tests for aquatic station revealed no significant difference for either observed richness or Shannon diversity ($W = 16.5$, $p = 0.498$ for both metrics). Similarly, fungal diversity did not differ among Protected Sites, Habitat Creation sites, or Intensive Grasslands for terrestrial samples (Kruskal–Wallis, $\chi^2 = 3.74$, $df = 2$, $p = 0.154$ for both metrics).

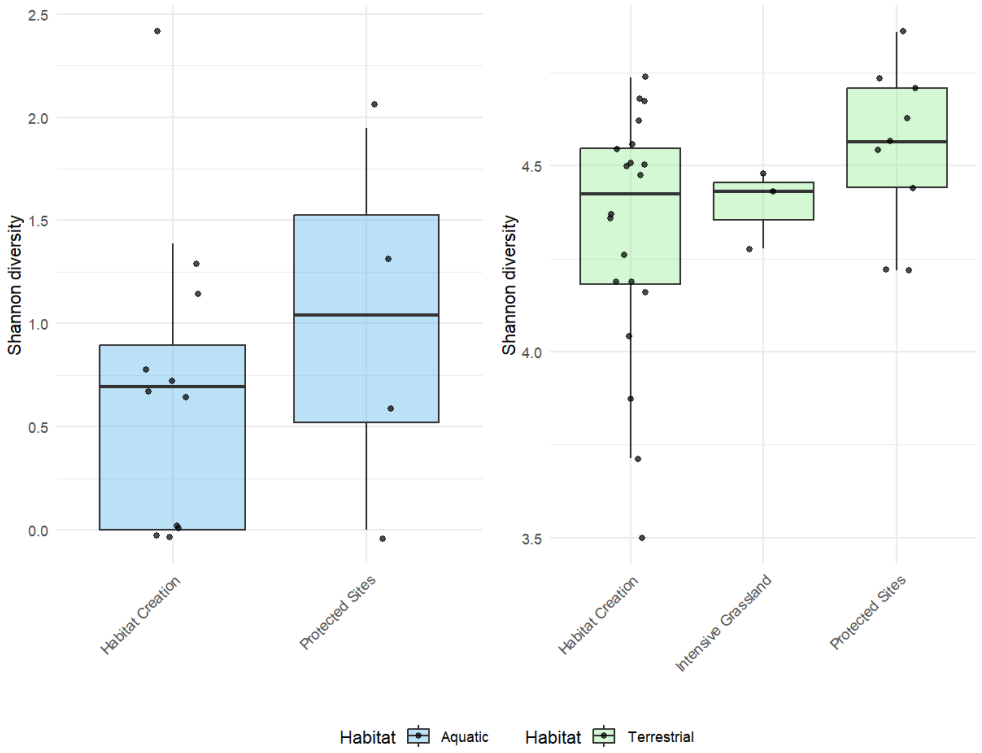

Figure 4: Differences in Shannon diversity by habitat station for aquatic (left) and terrestrial (right). Note the differences in scale between panels.

Ordination analyses showed clear differences in community composition across Conditions (Figure 5. For additional plots see supplementary document, section S2). Both NMDS and PCoA (Bray-Curtis and Jaccard matrices) showed separation between aquatic and terrestrial samples. Terrestrial samples were mostly distinct but clustered more tightly, while aquatic samples occupied a broader space with much overlap. PERMANOVA confirmed a significant effect on community composition for habitat (F = 8.52, R^2 = 0.159, p = 0.001), explaining ~16% of total variation within communities. For Conditions there was also a significant PERMANOVA effect (F = 3.39, R^2 = 0.244, p = 0.001), accounting for ~24% of community variation. The limited number of intensive grassland replicates make visual comparison difficult, but both the centroid and each sample site lie within the wildflower meadow ellipse. Station had a smaller but significant effect on community variation (F = 1.48, R^2 = 0.063, p = 0.032). However, betadisper tests indicated significant differences in multivariate dispersion for each PERMANOVA (table T3, supplementary document).

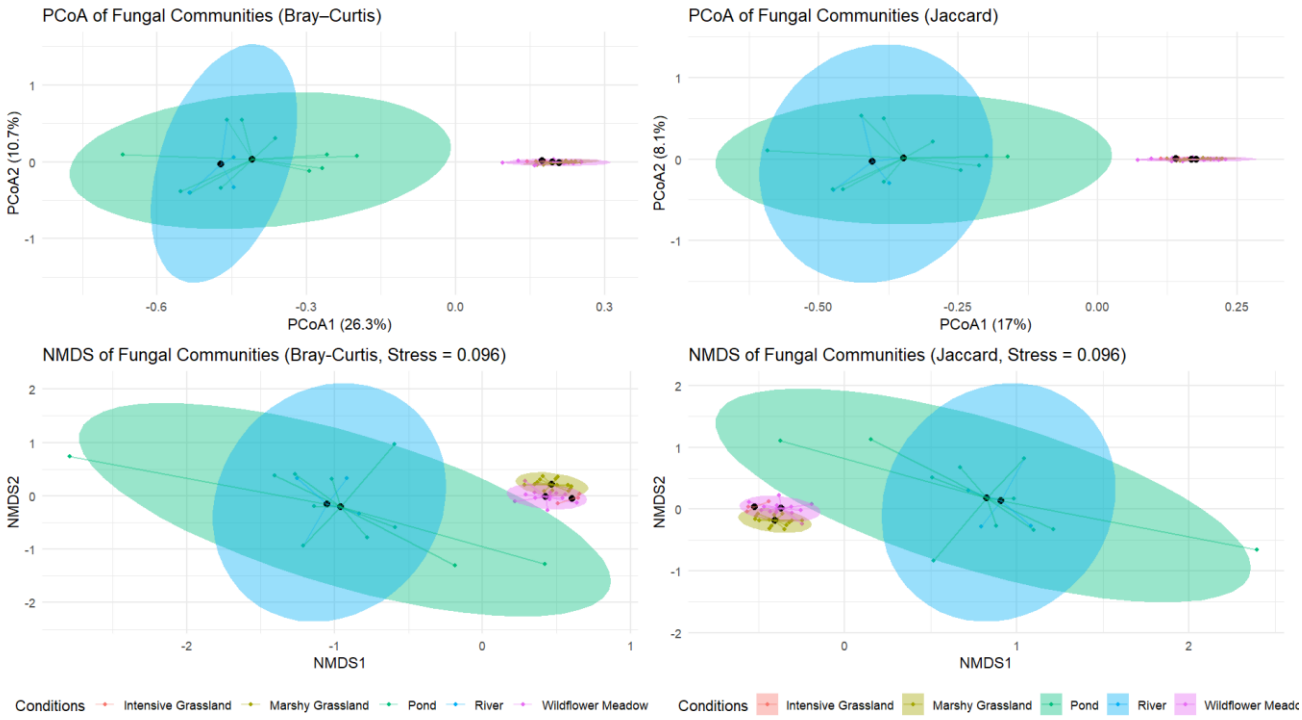

Figure 5: Faceted PCoA and NMDS plots using both Bray-Curtis and Jaccard dissimilarity matrices. Conditions are denoted by ellipse colour. Each sample site is represented by a corresponding colour point within an ellipse. Centroids are represented by back points.

4.2 Community composition and indicator species

FUNGuild analysis revealed marked differences in trophic composition between terrestrial and aquatic samples (Figures 6 - 7). Aquatic samples were dominated by pathotroph-saprotroph-symbiotrophs and pathotrophs, whilst terrestrial samples had a greater variety of trophic levels and were dominated by saprotrophs and pathotroph-saprotroph-symbiotrophs. In terrestrial samples, trophic mode composition was broadly similar across Habitat and Conditions, whereas aquatic habitat creation had a much higher proportion of pathotroph-saprotroph-symbiotrophs compared to the protected sites.

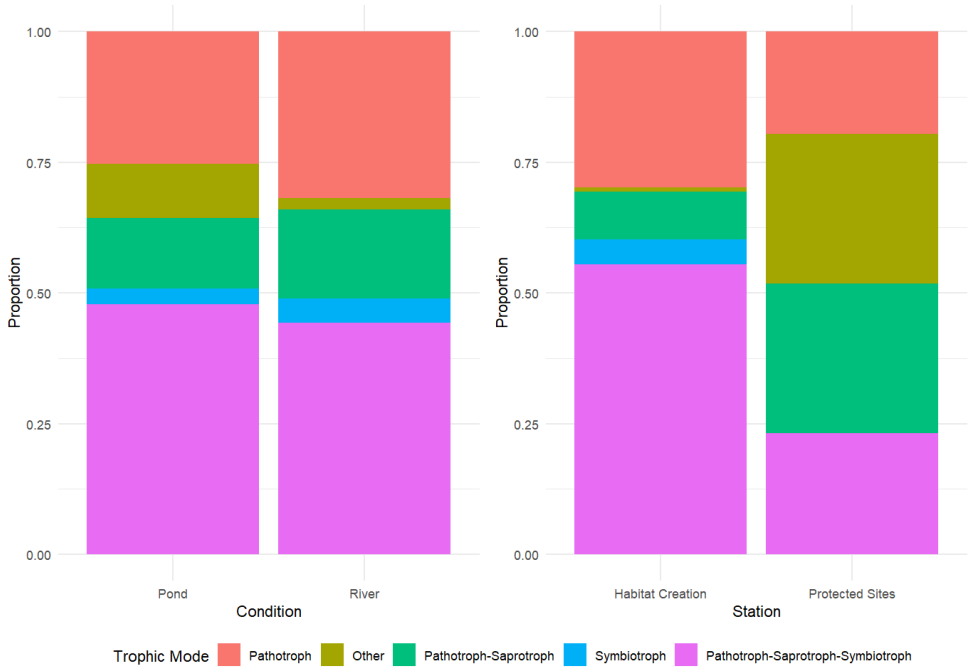

Figure 6: Proportional representation of trophic mode composition in aquatic samples by Condition (left) and Station (right). “Other” combines low proportion trophic modes, which included pathotroph-symbiotrophs, saprotroph-symbiotrophs, and saprotrophs.

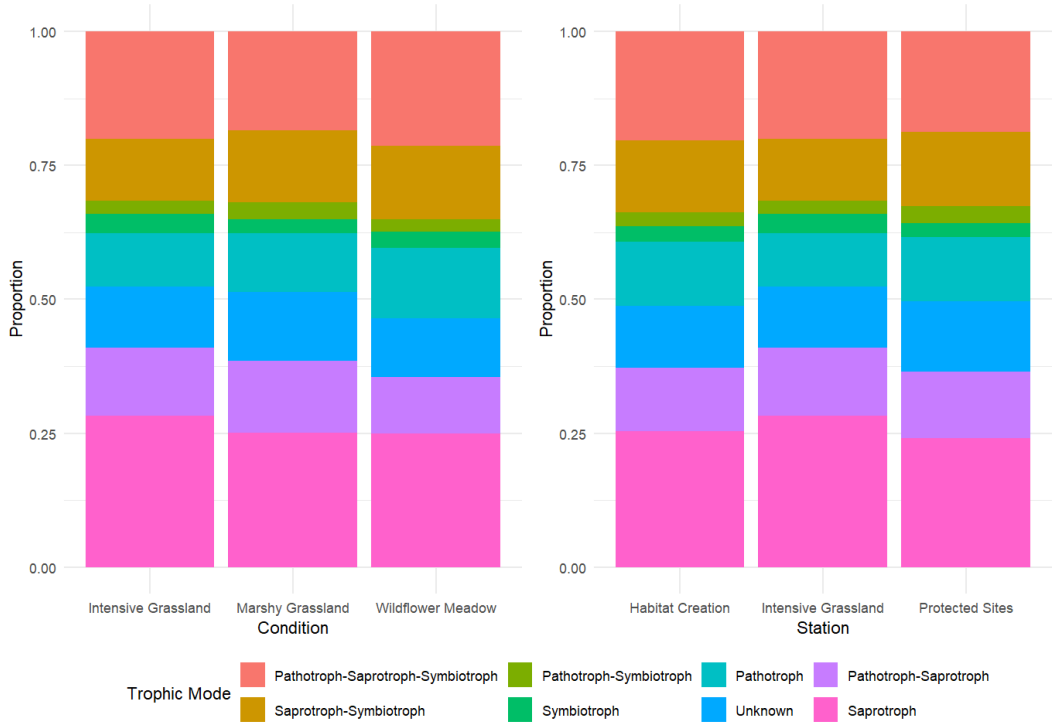

Figure 7: Proportional representation of trophic mode composition in terrestrial samples by Condition (left) and Station (right). The Unknown section denotes ASVs that were not recognised by FUNGuild.

Percentage stacked bar plots created at the phylum level revealed marked differences in phyla composition between aquatic and terrestrial samples. Aquatic habitats were dominated by Ascomycota, with Basidiomycota being the only other phylum present (Figure 8). In contrast, terrestrial habitats displayed a wider range of phyla, although Ascomycota and Basidiomycota were still the most dominant (Figure 9). For aquatic samples, Ascomycota were more pronounced in protected sites compared to habitat creation, whereas terrestrial samples typically displayed a similar proportion of phyla across both Condition and Station.

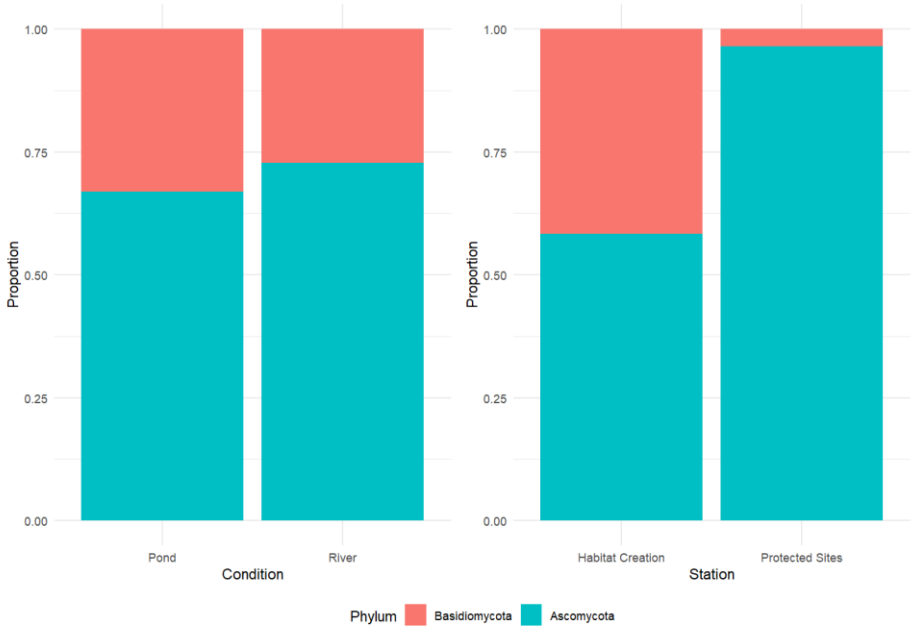

Figure 8: Proportional representation of fungal phyla composition in aquatic habitats by Condition (left) and Station (right).

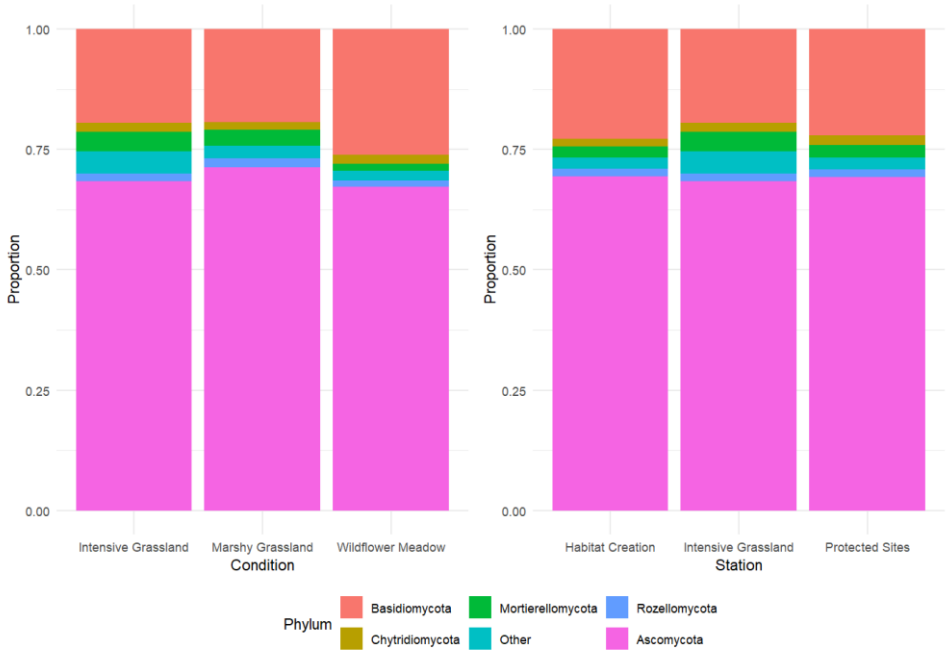

Figure 9: Proportional representation of fungal phyla composition in terrestrial habitats by Condition (left) and Station (right).

Indicator species analysis showed 36 significant ASVs associated with Condition and 31 significant ASVs associated with Station (tables T4 and T5, supplementary document). The Indicator Values (IndVal) were used to filter species with the highest IndVal statistic for plotting (Figure 10). Indicator species analysis showed no significant ASVs associated with aquatic habitats for both Condition and Station.

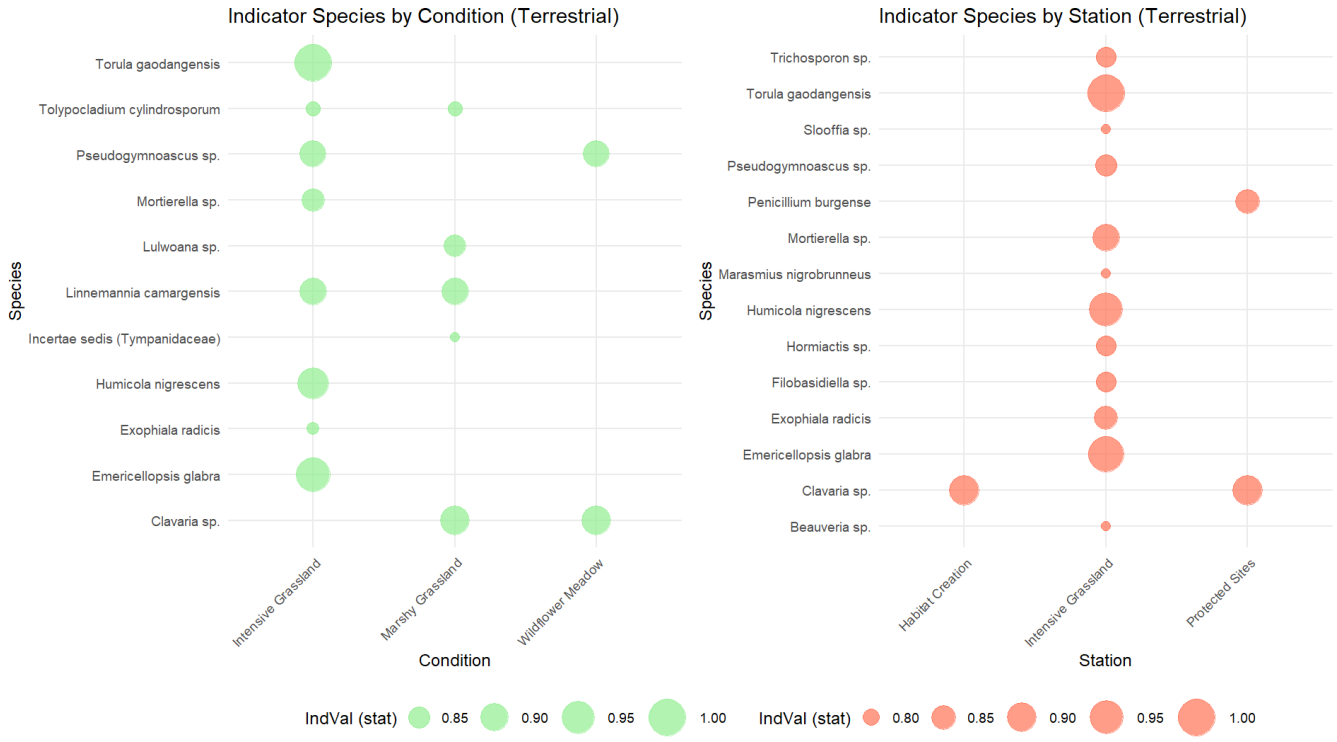

Figure 10: Data from ASV sequences with highest Indicator Values (IndVal) for both terrestrial Condition (left) and Station (right). The size of the point marks the IndVal, with larger points being closer to 1 and therefore more strongly associated with their respective habitats.

5. Discussion

5.1 Differences between aquatic and terrestrial fungi.

Terrestrial habitats are recognised as potential hotspots for fungal biodiversity due to their heterogeneous soils and abundant plant related substrates. Variations in nutrient availability, root exudates, pH, and soil moisture all contribute to diverse fungal communities and promote fungal plasticity and niche partitioning (Frąc et al., 2018). Aquatic habitats however are generally considered to host less fungal diversity, often attributed to reduced nutrient availability, lack of vegetation, and reduced structural complexity. As a result, aquatic systems are frequently overlooked as potential fungal habitats (Grossart et al., 2019).

In support of this, terrestrial habitats harboured significantly higher fungal diversity than aquatic habitats (Figure 2). The variety of vegetation in the terrestrial samples likely support many symbiotic associations. Indeed, terrestrial samples contained a total of 768 ASVs associated with symbiotrophic fungi (of 21,664 in total), compared to 360 (of 10,155 total) in aquatic habitats. However, many of the aquatic symbiotrophs were transient mycorrhizal associations from terrestrial areas that were likely blown or washed into the water from surrounding areas (Shearer et al., 2006).

Lower aquatic diversity reflects a more transient environment where fewer stable substrates occur, preventing long term fungal colonisation. Although some fungal species such as aquatic hyphomycetes carry out their entire life cycle in the aquatic environment, this is relatively uncommon (Pietryczuk, Cudowski and Hauschild, 2014). Despite this, it is estimated that only 7% of all aquatic fungal species have been identified and described meaning the actual richness of aquatic fungi may be substantially higher than the data suggests.

5.2 Condition did not shape terrestrial fungal diversity

Among terrestrial habitats, there were no significant differences in alpha diversity between the three Conditions despite expectations that intensive grasslands would host lower fungal diversity due to its reduced vegetation diversity, increased synthetic fertiliser application and increased soil disturbance: It is widely accepted that diverse vegetation promotes varied root exudates, increases litter substrates, and generally contributes to greater soil heterogeneity, all factors which should increase fungal diversity (Yang Y et al., 2017, Yang Z et al., 2021).

The non-significant result may reflect a reduction in statistical power due to substantially smaller sample size (n=3) compared to wildflower meadows (n=14) and marshy grasslands (n=15). However, additional practices such as the grazing that occurred on the intensive grasslands could have increased fungal biodiversity through disturbance; changes in soil structure and edaphic factors because of grazing would have increased soil heterogeneity, which could explain the high fungal diversity despite lower plant richness (Frąc et al., 2018). Abiotic factors (such as soil moisture, pH, and nutrient availability) may have also buffered differences in diversity: Even low diversity vegetation increases soil carbon content, influencing C:N ratios and indirectly supporting a higher fungal diversity (Cleveland and Liptzin, 2007, Maestre et al., 2015, Yang T et al., 2017).

There was also a variation in the amount of time that some habitat creation sites (which contain both wildflower meadows and marshy grasslands) had been under their current management techniques. For some of these sites, changes in land management practices were relatively recent, and it is likely that these sites had not yet developed the fungal communities typically associated with more established habitats. As a result, they would harbour a lower fungal diversity than expected for their assigned Condition, despite being grouped with the well-established sites. This variation in management history could also contribute to the lack of significant differences observed between the Conditions.

5.3 Community turnover, nestedness, and drivers of beta diversity

Ordination revealed strong separation in species composition between aquatic and terrestrial habitats, with terrestrial habitats forming distinct clusters associated with Condition, especially wildflower meadows and marshy grasslands (Figure 5). PERMANOVA supported these patterns, suggesting that Habitat and Condition were responsible for this separation. Although betadisper results were significant (part of the observed separation reflects unequal variability within groups as a result of both location and dispersion effects) location effects remain visually clear, especially in the terrestrial samples. The less significant PERMANOVA result for Station supports the hypothesis that fine-scale habitat features exert a stronger influence on fungal communities compared to broad land-management categories.

The marked distinction between wildflower meadows and marshy grasslands can be explained by species turnover and is consistent with the niche assembly theory, which states that environmental heterogeneity promotes niche differentiation and selects for specialised taxa (Webb et al., 2002). Therefore, community composition becomes increasingly different with environmental change and

niche differentiation (Wang et al., 2021). Because plant species and soil structure differ between these habitats, specialised niches arise, thus promoting divergence between the fungal communities.

In contrast, intensive grassland composition overlapped with both wildflower meadow and marshy grassland, which is characteristic of nestedness. Indicator species analysis reflects this, as many species characteristic of intensive grasslands were also indicators of richer habitats (Figure 10). This pattern suggests intensive grassland represents a reduced subset of the wildflower meadow and marshy grassland, consistent with patterns observed in degraded environments (Baselga, 2009, Wang et al., 2021). Additionally, the intensive sites are surrounded by both marshy grasslands and wildflower meadows and were historically similar habitats before being drained using land drains and ditches. As such, the intensive sites would have once formed part of a broader mosaic of comparable habitats, which helps explain the compositional overlap seen in the data. Aquatic communities also showed substantial overlap, likely reflecting nestedness caused by limited dispersal abilities and shared runoffs which introduce similar terrestrial fungi.

5.4 Community composition

FUNGuild analysis showed that aquatic systems were dominated by pathotrophs and other mixed pathotrophic modes (Figure 6). Such profiles, combined with the dominance of Ascomycota (Figure 8) often indicate pollution (Pietryczuk, Cudowski and Hauschild, 2014, Yan et al., 2018). This is consistent with surrounding agricultural land use and could explain the absence of fungi from several aquatic sample sites (n=10), especially if these runoffs contained fungicidal livestock parasiticides, run-off from slurry or manure application, run-off from synthetic fertiliser, septic tank discharge or sewage sludge run-off. Grazing intensity was also higher on habitat creation sites when compared to protected sites, with the potential for more dung (and any livestock veterinary medicines it contains) to be deposited in aquatic habitats on habitat creations sites than on protected sites.

In terrestrial habitats, trophic mode composition was more varied with saprotrophs and pathotrophs being the most dominant (Figure 7). This is consistent with high litter availability and the fungal composition of semi-natural grasslands where decomposition plays a major role in shaping microbial niches (Frąc et al., 2018). At the phylum level, Ascomycota again dominated, although the presence of Basidiomycota and a variety of other phyla were well represented (Figure 9). The absence of marked differences between terrestrial Condition was unexpected as Basidiomycota typically increase with vegetation recovery, and Ascomycete dominance declines in restored sites (Frąc et al., 2018). An assessment of vegetation health and biodiversity between terrestrial Conditions to identify areas potentially lacking in varied vegetation could help to explain a lower composition of Basidiomycota.

5.5 Limitations and Implications for biodiversity conservation

Although protected sites appeared to host more biodiversity than habitat creation (Figures 4,6 &7) these differences were not significant. This likely reflects unequal sample representation within station types but could also indicate that Condition has a stronger effect on fungal communities compared to broad land management techniques. Future sampling with even stratification would be necessary to clearly assess potential differences.

When interpreting these findings, it is important to consider several limitations. Namely, there were uneven sample sizes across Habitat, Condition, and Station, reducing statistical power. There was an absence of seasonal replication, which is important when considering fungi with several trophic modes depending on life stage. Furthermore, the dataset was not originally designed for fungal ecology questions and thus was inherently limited in its analysis. In future, a dataset containing even

sampling as well as additional biotic and abiotic factors would enable a more comprehensive analysis. For example, using existing data from National Vegetation Classification (NVC) Phase 2 vegetation surveys at each site would allow habitats to be characterised more precisely and would also identify plants commonly associated with certain species of fungi.

Despite these limitations, this study has demonstrated that eDNA metabarcoding detects clear ecological differences across Habitat and Condition. Results highlight the rich and functionally diverse fungal communities supported by wildflower meadows and marshy grasslands, evidencing their value in restoration and habitat creation. Aquatic fungal community composition proved sensitive to pollution, which further emphasises the importance of reducing agricultural runoffs and contamination from grazing livestock to prevent environmental stress.

In summary, these findings show that habitat heterogeneity, Condition, and agricultural runoff or contamination from grazing livestock strongly shape fungal communities, and demonstrate the value of eDNA metabarcoding with functional guild analysis as a more holistic assessment of biodiversity and restoration.

6. Acknowledgements

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7. References

Baselga, A. (2009). Partitioning the turnover and nestedness components of beta diversity. *Global Ecology and Biogeography*, 19(1), pp.134–143. doi: https://doi.org/10.1111/j.1466-8238.2009.00490.x

Cleveland, C.C. and Liptzin, D. (2007). C:N:P stoichiometry in soil: is there a 'Redfield ratio' for the microbial biomass?. *Biogeochemistry*, 85(3), pp.235–252. doi: https://doi.org/10.1007/s10533-007-9132-0

De Cáceres M, Legendre P (2009). "Associations between species and groups of sites: indices and statistical inference." Ecology, 90, 3566-3574. doi:10.1890/08-1823.1 https://doi.org/10.1890/08-1823.1

Frąc, M., Hannula, S.E., Bełka, M. and Jędryczka, M. (2018). Fungal Biodiversity and Their Role in Soil Health. *Frontiers in Microbiology*, [online] 9(707). doi: https://doi.org/10.3389/fmicb.2018.00707

Frøslev, T.G., Kjøller, R., Bruun, H.H., Ejrnæs, R., Hansen, A.J., Læssøe, T. and Heilmann-Clausen, J. (2019). Man against machine: Do fungal fruitbodies and eDNA give similar biodiversity assessments

across broad environmental gradients? *Biological Conservation*, 233, pp.201–212. Doi: https://doi.org/10.1016/j.biocon.2019.02.038

Grossart, H.-P., Van den Wyngaert, S., Kagami, M., Wurzbacher, C., Cunliffe, M. and Rojas-Jimenez, K. (2019). Fungi in aquatic ecosystems. *Nature Reviews Microbiology*, [online] 17(6), pp.339–354. Doi: https://doi.org/10.1038/s41579-019-0175-8

Li, N. and Dong, K. (2025). Fungal Communities in Various Environments. *Journal of Fungi*, 11(8), pp.560–560. doi: https://doi.org/10.3390/jof11080560

Maestre, F.T., Delgado-Baquerizo, M., Jeffries, T.C., Eldridge, D.J., Ochoa, V., Gozalo, B., Quero, J.L., García-Gómez, M., Gallardo, A., Ulrich, W., Bowker, M.A., Arredondo, T., Barraza-Zepeda, C., Bran, D., Florentino, A., Gaitán, J., Gutiérrez, J.R., Huber-Sannwald, E., Jankju, M. and Mau, R.L. (2015). Increasing aridity reduces soil microbial diversity and abundance in global drylands. *Proceedings of the National Academy of Sciences*, [online] p.201516684. doi: https://doi.org/10.1073/pnas.1516684112

McMurdie, P.J. and Holmes, S. (2013). phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE*, 8(4), p.e61217. doi: https://doi.org/10.1371/journal.pone.0061217

Nguyen, N.H., Song, Z., Bates, S.T., Branco, S., Tedersoo, L., Menke, J., Schilling, J.S. and Kennedy, P.G. (2016). FUNGuild: An open annotation tool for parsing fungal community datasets by ecological guild. *Fungal Ecology*, 20, pp.241–248. doi: https://doi.org/10.1016/j.funeco.2015.06.006

Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Borman T, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, Martino C, McGlinn D, Ouellette M, Ribeiro, Cunha E, Smith T, Stier A, Ter Braak C, Weedon J (2025). *vegan: Community Ecology Package*. R package version 2.7-2, https://CRAN.R-project.org/package=vegan

Pietryczuk, A., Cudowski, A. and Hauschild, T. (2014). Effect of trophic status in lakes on fungal species diversity and abundance. *Ecotoxicology and Environmental Safety*, 109, pp.32–37. doi: https://doi.org/10.1016/j.ecoenv.2014.07.032

Shearer, C.A., Descals, E., Kohlmeyer, B., Kohlmeyer, J., Marvanová, L., Padgett, D., Porter, D., Raja, H.A., Schmit, J.P., Thorton, H.A. and Voglymayr, H. (2006). Fungal biodiversity in aquatic habitats. *Biodiversity and Conservation*, 16(1), pp.49–67. doi: https://doi.org/10.1007/s10531-006-9120-z

Wang, Y., Lu, G., Yu, H., Du, X., He, Q., Yao, S., Zhao, L., Huang, C., Wen, X. and Deng, Y. (2021). Meadow degradation increases spatial turnover rates of the fungal community through both niche selection and dispersal limitation. *Science of The Total Environment*, 798, pp.149362–149362. doi: https://doi.org/10.1016/j.scitotenv.2021.149362

Webb, C.O., Ackerly, D.D., McPeek, M.A. and Donoghue, M.J. (2002). Phylogenies and Community Ecology. *Annual Review of Ecology and Systematics*, 33(1), pp.475–505. doi: https://doi.org/10.1146/annurev.ecolsys.33.010802.150448

Yan, D., Mills, J.G., Gellie, N.J.C., Bissett, A., Lowe, A.J. and Breed, M.F. (2018). High-throughput eDNA monitoring of fungi to track functional recovery in ecological restoration. *Biological Conservation*, 217, pp.113–120. doi: https://doi.org/10.1016/j.biocon.2017.10.035

Yang, T., Adams, J.M., Shi, Y., He, J., Jing, X., Chen, L., Tedersoo, L. and Chu, H. (2017). Soil fungal diversity in natural grasslands of the Tibetan Plateau: associations with plant diversity and productivity. *New Phytologist*, 215(2), pp.756–765. doi: https://doi.org/10.1111/nph.14606

Yang, Y., Dou, Y., Huang, Y. and An, S. (2017). Links between Soil Fungal Diversity and Plant and Soil Properties on the Loess Plateau. *Frontiers in Microbiology*, 8. doi: https://doi.org/10.3389/fmicb.2017.02198

Yang, Z., Shi, Y., Wang, J., Wang, L., Li, X. and Zhang, D. (2021). Unique functional responses of fungal communities to various environments in the mangroves of the Maowei Sea in Guangxi, China. *Marine Pollution Bulletin*, 173, p.113091. doi: https://doi.org/10.1016/j.marpolbul.2021.113091