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Fungal Diversity in a Mountain Lake Is Explained by Weather Patterns

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ABSTRACT

1. Fungi are an important component of aquatic ecosystems because of their multifaceted roles such as organic matter decomposition, nutrient cycling and energy transfer to higher trophic levels. Despite their importance, little is known about the diversity and ecological functions of fungi in mountain lakes in general and specifically for different layers.
2. We investigated the spatiotemporal distribution of fungi in mountain Lake Tovel (Northern Italy) over more than 3 years (September 2021 to February 2025) using metabarcoding (amplicon sequence variants—ASVs—of the ITS gene). We sampled the upper and lower euphotic layers and the hypolimnion of the deep basin and the surface and bottom of the shallow basin. The sampling period covered the year 2022, one of the hottest in Europe, and the year 2024, a very rainy year in Northern Italy.
3. Fungi showed year differences in % read abundance at different taxonomic levels. Observed richness and Chao1 diversity of the upper and lower euphotic layer were lower in warm 2022 than rainy 2024. Basidiomycota showed lower mean % read abundance in 2022 than 2024 in the intermediate layer. In the lower euphotic layer and the hypolimnion, most fungal classes (one and six fungal classes, respectively) had a lower % read abundance in 2022 than 2024. In NMDS with ASVs (stress = 0.16), samples of the years 2022 and 2024 were generally separated. In a partial redundancy analysis conditioned on layers, 17.2% of ASV variability was explained by year ($p < 0.001$) and season ($p < 0.001$) as factors and pH ($p < 0.01$) and P_{tot} ($p < 0.05$) as continuous variables. In path analysis, precipitation and % DO directly increased and pH directly decreased fungal diversity. The % abundance of dominant fungal genera also varied by layer: *Filobasidium* and *Rhodotorula* had higher % read abundance in the hypolimnion than lower euphotic layer while *Zygophlyctis* showed the opposite pattern. In network analysis, the lower euphotic layer showed the highest topological complexity, indicative of robustness against perturbations. The interaction between *Zygophlyctis* (primary algal parasite) and *Cystobasidium* (mycoparasite) pointed to hyperparasitism in the lower euphotic layer.
4. Year differences in fungal communities indicated that the higher diversity of fungal communities in rainy 2024 can be attributed to different aspects such as wash-in of fungi and of allochthonous material by rain. Season and layer differences were weak and related to specific analyses and genera and phyla.
5. Our study provided new information on the diversity of fungal communities occurring in mountain lakes. Fungal communities responded to altered weather patterns, an important aspect considering that ongoing climate change alters precipitation in mountain regions.

1 | Introduction

Fungi occur almost everywhere and link organisms to ecosystems (Bahram and Netherway 2022). Despite this importance, the diversity of fungi and their ecological and biogeochemical roles are much less understood in aquatic than in terrestrial ecosystems (Grossart et al. 2019; Debeljak and Baltar 2023). Aquatic fungi interact with organisms at all levels: fungi inhibit bacterial growth, are both predators and prey of heterotrophic protists, are saprotrophs, are parasites of phyto-, zooplankton, of other fungi, of higher organisms such as frogs and are food for aquatic invertebrates (Grossart et al. 2019). These complex interactions play a vital role in freshwater lakes, contributing to nutrient transformation, organic matter decomposition and carbon cycling, and have led to the introduction of the 'mycoflux', referring to fungal interactions affecting the aquatic carbon pump (Grossart et al. 2019). Parasitic chytrids (phylum Chytridiomycota) favour the edibility of phytoplankton and thus support zooplankton production against the impacts of eutrophication and warming. This relationship led to the formulation of the chytrid insurance hypothesis (Abonyi et al. 2024), further pointing to the importance of fungi for the functioning of lake ecosystems.

Fungi occur in a variety of habitats across seasonal and environmental gradients. Chytridiomycota are more abundant in lakes than rivers (Lepère et al. 2019) and show a high diversity similar to that of the dominant autotrophic groups found in mountain lakes (Ortiz-Álvarez et al. 2018). In mesotrophic Lake Stechlin (Germany), parasitic and saprotrophic Chytridiomycota dominate in spring while Ascomycota and Basidiomycota dominate during autumn and winter (Van den Wyngaert et al. 2022). Lake sediments of Antarctica lakes (de Souza, Lirio, et al. 2022) and surface waters of three Chinese plateau lakes (Fang et al. 2025) are dominated by saprotrophic fungi. In eutrophic lakes in Poland, the epilimnion and metalimnion show highest fungal abundance while the epilimnion and hypolimnion show highest species diversity (Cudowski and Pietryczuk 2020). In oligo-mesotrophic Lake Pavin (France), the fungal community shows a homogenous spatial distribution while in eutrophic Lake Aydat (France) a more heterogenous distribution (Monchy et al. 2011). In spring, fungal communities are related to salinity, total organic carbon, total nitrogen and ammonium in two meromictic hypersaline lakes (Mircea et al. 2024). Across seasons, total organic carbon and dissolved oxygen are linked to the fungal community composition of boreal and subarctic lakes (Sanyal et al. 2025). Thus, fungal communities show communalities and differences between lakes related to environmental conditions.

While the interest in the diversity and ecological functions of fungi is increasing (Isola and Prenafeta-Boldú 2025), little is known about these topics for mountain lakes (de Souza, Convey, et al. 2022). We investigated the spatiotemporal distribution of the fungal community in mountain Lake Tovel (Northern Italy) over more than 3 years (September 2021 to February 2025) covering different weather conditions. The year 2022 was one of the hottest in Europe (Ballester et al. 2023) and the year 2024 showed above-average precipitation in different areas such as Northern Italy (Copernicus Climate Change Service and World Meteorological Organization 2025). Warming generally leads to changes in a lake's thermal habitat (Kraemer et al. 2021) and specifically in Lake Tovel, cold spells and heavy rain induce lake mixing leading to the oxygenation of the hypolimnion (Flaim et al. 2020). Apart from providing baseline information on fungal diversity in a mountain lake, we hypothesised that fungal community composition would differ between 2022 and 2024 given their different environmental conditions. The bacterioplankton of Lake Tovel shows distinct communities in the littoral, pelagic and hypolimnion despite within-lake dispersal by water flow and water mixing in autumn (Obertegger et al. 2018). We further hypothesised that distinct fungal communities would occur in different layers of the lake. Our study, therefore, aimed to shed light on fungal diversity and environmental influences in a mountain lake, an understudied habitat.

2 | Methods

2.1 | Study Site

Lake Tovel (46.26137° N, 10.94934° E; altitude: 1177 m above sea level; area: 0.4 km^2; maximum depth: 39 m) is an Italian long-term ecological research site (LTER_EU_IT_090) in the Brenta Dolomites and belongs to international networks (LTER-Europe and ILTER). The lake is oligotrophic (annual mean values over the whole water column: total dissolved phosphorus [P_{tot}] < 10 $\mu g\,L^{-1}$, dissolved organic carbon < 1 $mg\,L^{-1}$, conductivity ~180 $\mu S\,cm^{-1}$; coefficient of light attenuation ≤ 0.18 and water transparency > 10 m; depth layer surface to 20 m depth: chlorophyll-a < 3 $\mu g\,L^{-1}$; Cellamare et al. 2016) and is surrounded by a spruce forest mixed with *Fagus sylvatica* and *Abies alba* (Gottardini et al. 2004).

2.2 | Sampling and Environmental Variables

Other studies sample early in the year to avoid the overrepresentation of airborne spores of wood-degrading Basidiomycetes originating from the surrounding forest (Wurzbacher et al. 2016), or avoid sampling after rain to solely characterize soil and freshwater fungi (Sieber et al. 2020). We were interested in the whole fungal diversity present in the lake, irrespective of their origin (out of lake versus indwellers). Samples for fungal metabarcoding were taken monthly over the deepest part of the shallow and deep basin from September 2021 to February 2025 ($n = 84$). The upper (0–3 m; A0-3) and lower euphotic layer (3–25 m; A3-25) and the deep hypolimnion (30–35 m; A35) of the deep basin and the surface (0 m; B0) and bottom (4 m; B4) of the shallow basin were sampled according to Obertegger (2022). We used these layer abbreviations for their easier reference in the result section. For each sampling, water temperature and percent dissolved oxygen saturation (% DO) were measured with a multiparametric probe (Idronaut Ocean Seven 316 Plus) at 1-m intervals, and values were averaged for the respective layers. Water samples for chemical analyses (pH, total nitrogen [N_{tot}], P_{tot}, silica) were taken with a bottle from specific depths (surface, at a depth of 1 m, and then at 5-m intervals). Chemical analyses were performed according to American Public Health Association et al. (2023), and values were averaged for the respective layers. Chlorophyll-a (chl-a) was assessed for A0-3, A3-25 and A35 by the trichromatic method (American Public Health Association et al. 2023). Chl-a profiles were taken fluorometrically with a

FluoroProbe II (bbe-Moldaenke, Kiel, Germany) at several sampling occasions over the deepest basin.

We used the nearest weather station (Cles: 656 m a.s.l.; 46.361 N, 11.040 E; 13 km north of Lake Tovel; hourly recordings) to characterize different weather patterns (precipitation, air temperature).

Data management, analyses and plotting were performed with R version 4.5.1 (R Core Team 2025).

2.3 | DNA Extraction, Sequencing and Analysis of ITS rRNA Gene Sequences

We gently vacuum filtered water samples of up to 1.5 L or until the filter was clogged onto sterile 0.2 μm membrane filters (Supor 200 Membrane Disc Filters, 47 mm; Pall Corporation, East Hills, NY, USA) for environmental DNA (eDNA) extraction of fungi (particle attached and free-living). Filters were stored at −80°C until extraction with the PowerWater DNA isolation kit (MOBIO Laboratories Inc., CA, USA). eDNA was PCR-amplified by targeting the ITS1 rRNA with overhanging Illumina adapters. We used the fungal primer ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′; Gardes and Bruns 1993) and ITS1R (5′-GCTGCGTTCTTCATCGATGC-3′; White et al. 1990) that are universal fungal primers and are commonly used in fungal community analyses of soil (Buée et al. 2009) and freshwater (Monchy et al. 2011). Amplicon library preparation was performed using a two-step amplification protocol with the FastStart High Fidelity PCR System (Roche). Each sample was amplified in a 25 μL PCR reaction containing 2.5 μL of 10× FastStart High Fidelity Reaction Buffer (Roche), 0.25 μL of FastStart High Fidelity Enzyme Blend (5 U μL^{-1}; Roche), 1 μL of each primer (10 μM) and 3 μL of template DNA (5–20 ng μL^{-1}). PCR reactions were carried out in a GeneAmp PCR System 9700 (Thermo Fisher Scientific) at the following cycling conditions: an initial denaturation at 95°C for 3 min; 30 cycles at 95°C for 20 s, 50°C for 45 s and 72°C for 90 s; followed by a final extension at 72°C for 10 min. Finally, sequencing was performed on an Illumina MiSeq platform using the MiSeq Reagent Kit v3 (600-cycle, PE300).

Amplicon sequence variants (ASVs) were obtained using package DADA2 (Callahan et al. 2016, 2017) following recommendations in the DADA2 tutorial (https://benjjneb.github.io/dada2/ITS_workflow.html) and Rolling et al. (2022). ASVs were classified using the UNITE database (Nilsson et al. 2019), and unclassified ASVs were aligned against the filtered NCBI non-redundant nucleotide sequences (nt) database (October 2025) using BLASTn with default parameters. The nt database was filtered using the following keywords: 'ITS1', 'Internal transcribed spacer' and 'internal transcribed spacer' similarly to de Souza, Lirio, et al. (2022).

Rare ASVs are often removed as potential artefacts (Bálint et al. 2016) at the proposed threshold from 1 to 10 reads across all samples (Brown et al. 2015). We excluded sequences with < 5 reads as a compromise between keeping rare ASVs and deleting artefacts similar to Debeljak and Baltar (2023). The ASV table was transformed by rarefaction, and data were expressed as percentage (% read abundance; from here onwards % abundance) with respect to the total number of reads. Indwellers, periodic immigrants and versatile immigrants are present in fungal communities (Grossart et al. 2019). We considered ITS sequences as a fingerprint of total fungal biodiversity and referred to the total community as mycoplankton (Gauthier et al. 2025).

The ITS dataset showed an uneven distribution of samples for years and layers (Table S1) because of the generally restricted access to the lake during winter (only one under-ice sampling per winter, no under-ice sampling at the shallow basin that is generally dry during winter), failed sequencing, and no access to the lake from September to December 2024 because of a landslide blocking the road.

2.4 | Data Analysis

We used package headwaveR (Schlegel and Smit 2018) to find periods of heat and cold spells considering the last 12 years (2014–2025) of air temperature. Heat spells (cold spells) were identified as anomalous warm (cold) events during which temperatures were warmer (colder) than the 90th percentile (10th percentile) of the 12-year baseline period for 5 days or more (Hobday et al. 2018).

When referring to years, under-ice data were merged with samplings of the preceding year (e.g., under-ice sampling of the year 2023 was related to the ice-free samplings of the year 2022; Table S1) like in Obertegger (2022) and Obertegger et al. (2025), because we accordingly reasoned that samples of the preceding year are linked to under-ice conditions of the following year.

To characterize year differences of environmental variables, we considered all monthly data, also those for which no eDNA data were available. We used non-parametric one-way ANOVA and post hoc testing corrected for multiple testing to assess year differences in environmental variables (monthly mean air temperature; water: temperature, pH, nutrients). We used Cohen's D (D; package effectsize; Ben-Shachar et al. 2020) as a metric for the *magnitude of year differences* and only reported significant differences ($p < 0.05$) representing medium ($0.5 < D < 0.8$) or strong effects ($D > 0.8$; Sullivan and Feinn 2012). In ANOVA, we did not consider samples of the year 2021 because of their limited number (Table S1).

Year and layer differences in mean % abundance and alpha diversity indices (observed richness, Shannon diversity, Chao1 index accounting for species that are likely present but were not detected) were assessed by non-parametric one-way ANOVA, post hoc testing corrected for multiple testing, and calculating D, using the same thresholds as for environmental variables. In ANOVA, % data were arcsine-transformed and the Kruskal–Wallis test was used to relax the assumption of equal variances between groups. For yearly mean data, we reported mean values ±1 standard deviation. We refrained from performing two-way ANOVA and using any interaction term, considering the uneven and low number of samples per year and layer (Table S1).

We used the analysis of multivariate homogeneity of group dispersion to assess year, season and layer differences (package

vegan; Oksanen et al. 2025; function betadisper). We used Bray–Curtis dissimilarity to partition the dissimilarity matrix between years, seasons, and layers (PERMANOVA; package vegan; Oksanen et al. 2025; function adonis). Significant results from a PERMANOVA can be related to significant differences in the variability between groups; thus, non-significant results from betadisper corroborate PERMANOVA. In both analyses, samples of the year 2021 and of the under-ice seasons were excluded because autumn samples of 2021 were not enough to represent yearly variability and under-ice samplings were few with respect to other seasons (Table S1).

We applied non-metric multidimensional scaling (NMDS) with all samples and used environmental fitting to link environmental variables to the ordinations. In NMDS, we added ellipses that group communities according to statistically significant factors (year, season and layer). Furthermore, we performed a redundancy analysis (RDA; package vegan; Oksanen et al. 2025) with Hellinger-transformed abundance data to stabilize the variance and account for the high number of zeros (Legendre and Gallagher 2001). We excluded ASVs that were present in $<10\%$ of samples and had <10 reads. We used a partial RDA (pRDA) conditioned on layer to account for layer-independent environmental effects and applied forward and backward selection to find the most parsimonious environmental variables explaining ASV variability. To further inspect the partial model, we tested for significant interactions between environmental variables and layer. To quantify the relative contribution of environmental versus spatial layer effects, we used variance partitioning. In the pRDA, the top 10 ASVs with the highest affinity on the selected environmental variables were assessed.

We used the analysis of composition of microbiomes (ANCOM; package ANCOMBC; Lin and Peddada 2020) for drawing inferences regarding taxon abundance in different years. ANCOM has good control of the false discovery rate and is very sensitive for >20 samples per group (Weiss et al. 2017). We merged samples of the same years across layers to have enough statistical power.

We used network analysis (packages microeco; Liu et al. 2021; package igraph; Csárdi et al. 2026) to explore microbial interactions. We focused on layers of the main basin having between 17 and 21 samples to ensure a robust network construction. We filtered rare taxa with a relative abundance $<0.1\%$ to reduce spurious Spearman's rank correlations. Only significant ($p<0.05$) correlations >0.6 were included in the final network. We used network metrics (clustering coefficient, density, modularity) to characterize structural complexity. Network topology (modularity, clustering) was compared to a null-model ($n_{\text{permutation}}=999$) to assess the significance of the module structure (Csárdi et al. 2026). Network modules were identified using the fast greedy modularity optimization algorithm. Keystone taxa (hubs) were identified based on the number of direct connections (degree; major hubs: degree >6) and betweenness centrality. We utilized the FungalTraits database (Põlme et al. 2020) for functional annotation of ASVs (hubs). Layer differences in the relative abundance of key hub taxa were tested using the Kruskal–Wallis test followed by Wilcoxon rank-sum tests for pairwise comparisons.

We used path analysis, a subset of structural equation modelling (package lavaan; Rosseel et al. 2025; package tidySEM; van Lissa 2026) to test the influence of environmental variables on fungal diversity (taxa richness, Chao1). Our model simultaneously estimated the effect of air temperature (monthly mean) and precipitation (total sum until sampling) as exogenous predictors on fungal diversity and on potential mediators (% DO, silica, pH). Direct paths from the climate variables to fungal diversity were included to account for residual effects, and covariances between mediators were estimated to ensure a robust model structure (Data S1). Path coefficients were estimated using maximum likelihood estimation, and we applied non-parametric bootstrapping ($n=1000$) to assess the significance of indirect effects. All reported coefficients were standardized to allow for a direct comparison of effect strengths across different environmental variables. The model estimated 21 parameters based on 83 samples across layers (one sample from A0-3 was excluded because of missing silica).

3 | Results

3.1 | Weather Conditions

With respect to the last 12 years, heat spells were found in June 2021, May and July 2022, August and October 2023, and February 2024. Cold spells were found in November 2021, April 2021 and April 2024 (Table S2). The year 2022 was generally the driest and the year 2024 was the rainiest during the sampling period with frequent rain in November 2023 (Figure 1A; day of the year 305–334). Monthly mean air temperature was generally highest from June to August (Figure 1B). Monthly mean air temperatures during May ($D=0.97$; non-parametric one-way ANOVA: $p<0.001$), June ($D=0.97$; $p<0.001$) and July ($D=0.75$; $p<0.001$) were higher in 2022 than in 2024, while in August ($D=0.53$; $p<0.001$) monthly mean air temperature was higher in 2024 than 2022 (Figure 1B).

3.2 | In Situ Environmental Variables

In most layers, mean water temperature was generally highest during summer (June to September) 2022 and lowest during summer 2024 (Figure 2A,B). Contrarily, mean water temperature in A35 was higher during August and September of 2024 than in 2022 (Figure 2A).

The mean % DO in A0-3 (range April to December: 90%–122%; Figure 2C) did not show marked year differences. Contrarily, the mean % DO in A3-25 was lower from April to October in 2021 and 2024 compared to 2022 and 2023 even though values remained constantly high ($>80\%$ DO%; Figure 2C). In A35, mean % DO was substantially higher ($>40\%$ DO) in 2024 compared to the other years (range 0%–30% DO%; Figure 2C). In the shallow basin, % DO varied from 95% to 120% in B0 and from 75% to 150% in B4 (Figure 2D).

Yearly mean N_{tot} and P_{tot} concentrations did not show any year differences in all layers. Contrarily, yearly mean pH was higher in 2022 than 2023 and 2024 in all layers except for A35 (Table S3). Yearly mean silica concentrations were higher in

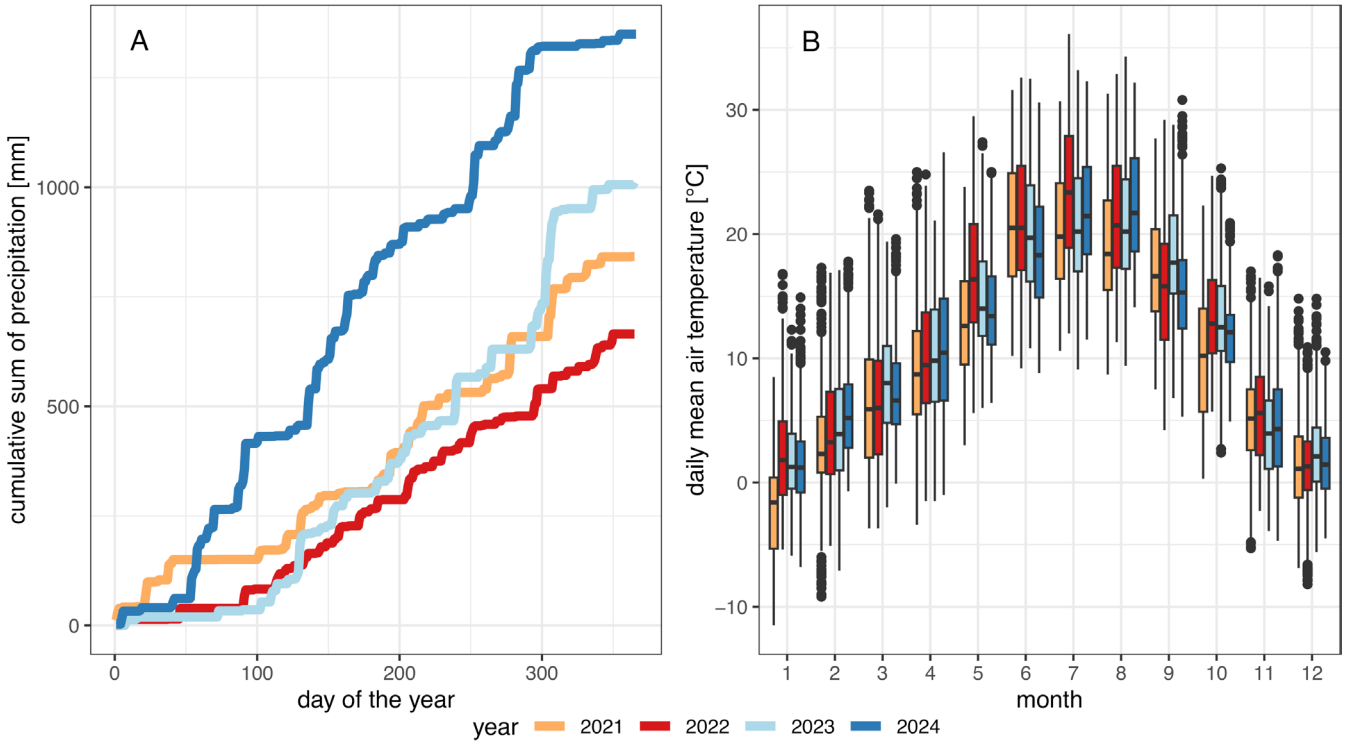

FIGURE 1 | Temporal evolution of precipitation and air temperature: (A) cumulative sum of precipitation; (B) boxplots of daily mean air temperature; numbers indicate months.

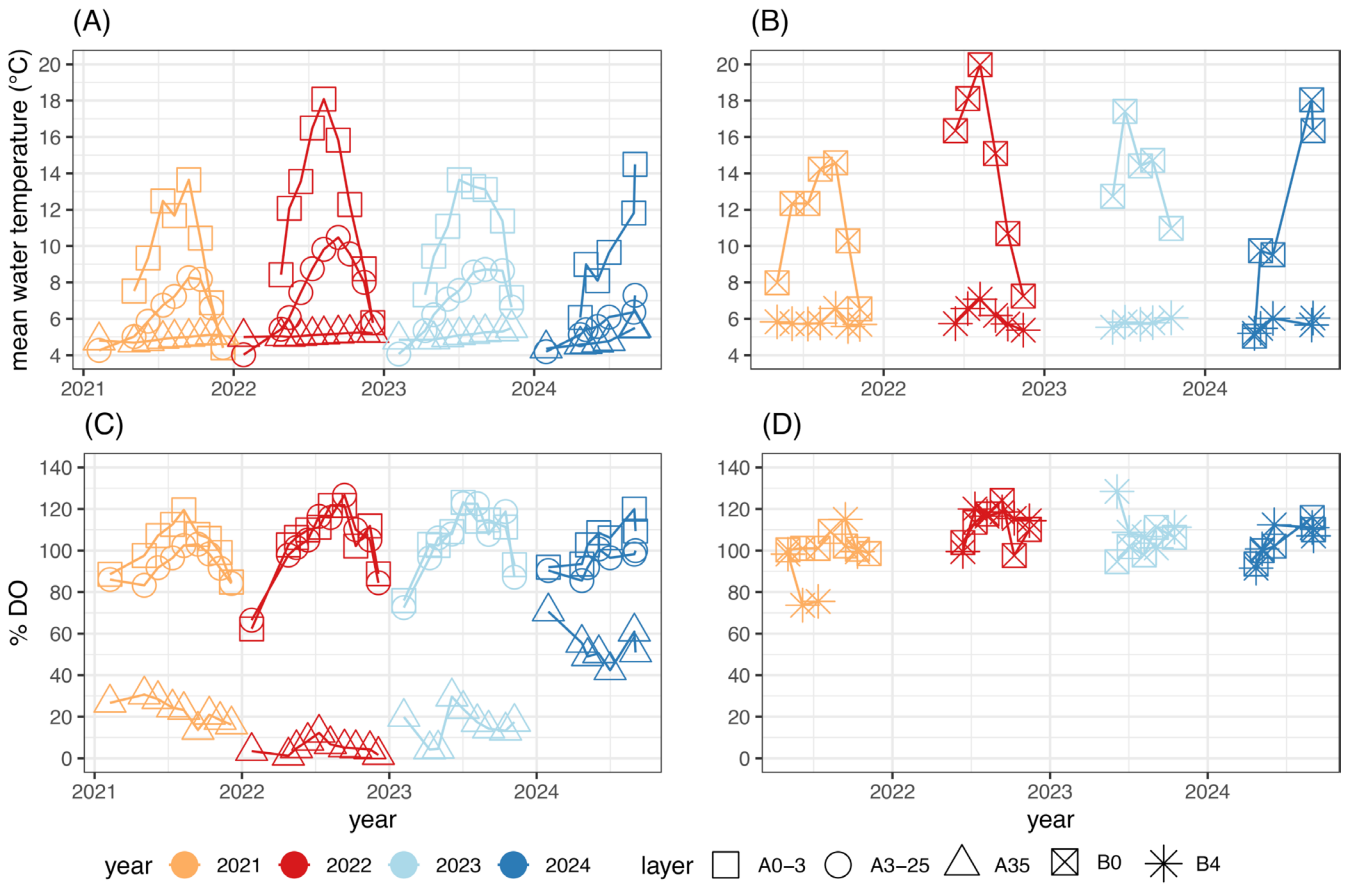

FIGURE 2 | Temporal monthly pattern of mean water temperature (A, B) and % DO (C, D) of the different layers and years; (A, C) deep basin: upper euphotic layer—A0-3; lower euphotic layer—A3-25; deep hypolimnion—A35; (B, D) shallow basin: surface—B0; bottom layer—B4.

2022 than 2023 ($p < 0.05$; $D = 0.9$) and 2024 ($p < 0.01$; $D = 1.5$) in A35 (silica$_{2022}$: $2.6 \pm 0.97\,mg\,L^{-1}$; silica$_{2023}$: $1.9 \pm 0.49\,mg\,L^{-1}$; silica$_{2024}$: $1.5 \pm 0.16\,mg\,L^{-1}$).

Yearly mean chl-a concentrations did not differ significantly between A0-3 and A3-25 while concentrations were higher ($p < 0.01$; $D = 1.74$) in 2022 ($3.7 \pm 0.77\,\mu g\,L^{-1}$) than in 2024 ($2.2 \pm 0.91\,\mu g\,L^{-1}$) in A35.

As indicated by Fluoroprobe profiles, chl-a peaked at deeper depths (May$_{2022}$: 30 m; June$_{2022}$: 27 m; July$_{2022}$: 25 m) during 2022 than during 2024 (May$_{2024}$: 25 m; June$_{2024}$: 21 m; July$_{2024}$: 20 m; Figure S1). Furthermore, marked deep chl-a maxima were found at 27 m at 28 m in August and in September 2022, respectively. Chl-a peaks of 2023 (May$_{2023}$: 28 m; June$_{2023}$: 29 m; July$_{2023}$: 27 m; July$_{2023}$: 25 m; August$_{2023}$: 24 m) were similarly deep as that of 2022 in accordance with the second-highest water temperatures in 2023.

3.3 | Fungal Diversity

For most samples, species richness saturated at an abundance of 10,179 reads (Figure S2), the threshold for rarefying samples. After rarefying, 4925 ASVs were found in total. On the average per layer, 3%–6% of ASVs were not identified at class level, 7%–9% not at order level, 12%–15% not at family level, 16%–17% not at genus level and 53%–60% not at species level.

In total, 11 fungal phyla were found. Seven phyla (Ascomycota, Basidiomycota, Chytridiomycota, Rozellomycota, Aphelidiomycota, Mortierellomycota, Mucoromycota) and Fungi incertae sedis occurred in all layers. Phylum Blastocladiomycota was not found in A0-3, phylum Olpidiomycota was only found under ice 2022 in A3-25 and during May and June 2022 in A35, and phylum Basidiobolomycota was only found during June 2024 in A35. Ascomycota, Basidiomycota and Chytridiomycota were the dominating phyla in all layers (Figure S3).

In A0-3, B0 and B4, no phylum showed significant year differences in mean % abundance. In A3-25, % abundance of Basidiomycota was higher ($p < 0.05$; $D = 1.6$) in 2024 ($15\% \pm 10.3\%$) than in 2022 ($14\% \pm 28.1\%$). Contrarily in A35, % abundance of Basidiomycota was higher ($p < 0.01$; $D = 2.9$) in 2023 ($42\% \pm 13.5\%$) than in 2024 ($15\% \pm 10.4\%$). Among layer comparisons, % abundance of Ascomycota was higher ($p < 0.05$; $D = 0.94$) in A35 ($41\% \pm 22.2\%$) than in A3-25 ($21\% \pm 20.5\%$) while % abundance of Chytridiomycota was lower ($p < 0.01$; $D = 1.23$) in A35 ($27\% \pm 28.4\%$) than in A3-25 ($63\% \pm 29.9\%$; Figure S3).

Of the 53 fungal classes found, 34 occurred in all layers. Class Sporobolomyces (Basidiomycota) and Blastocladiomycota class incertae sedis were unique to A3-25. Classes GS18 and GS37 (both Olpidiomycota), Mucoromycetes (Mucoromycota) and Basidiobolomycetes (Fungi phylum incertis sedis) were unique to A35. Fourteen fungal classes showed at least once a % abundance greater than or equal to 4% (Figure 3). In A3-25 and A35, one and six fungal classes, respectively, showed year differences in mean % abundance. Most of them showed higher values in 2024 than 2022 and 2023 (Table 1). Only Cystobasidiomycetes and Microbotryomycetes showed higher values in 2022 and 2023, respectively, than in 2024 (Table 1; Figure 3). Microbotryomycetes, Sordariomycetes, Eurotiomycetes and Tremellomycetes showed higher values in A35 than A3-25 while Chytridiomycetes showed higher values in A3-25 than A35. Only Eurotiomycetes showed basin differences, with B0 showing higher % abundance than A3-25 (Table 2; Figure 3).

Of the 935 identified genera, 210 (22.5%) occurred in all layers at least once. Within the deep basin, more genera were in common between A0-3 and A3-25 (42.0% in common) than between A35 and A0-3 (37.7%) and between A35 and A3-25 (34.0%). Within the shallow basin, only 36% of genera were in common between B0 and B4. Less than 9% ($n_{A0\text{-}3} = 74$; $n_{A3\text{-}25} = 51$; $n_{A35} = 64$; $n_{B0} = 84$; $n_{B0} = 51$) were unique to layers.

Of the top 10 genera with highest mean % abundance in each layer (Table S4), *Cladosporium*, *Filobasidium*, *Rhodotorula* and *Zygophlyctis* showed highest abundance in all layers, while *Emericellopsis* and *Exophiala* showed highest abundance only in A0-3, *Leptobacillium* only in A3-25, *Neocucurbitaria* only in A35, *Alternaria* only in B0 and *Sporidiobolus* only in B4. To investigate meaningful layer differences, we focused on the genera with highest abundance and occurring in all layers. *Filobasidium*, *Rhodotorula* and *Zygophlyctis* showed layer differences mostly between A3-25 and A35, while *Cladosporium* did not show any layer differences (Table 3).

Among the top 10 genera specific for each layer, *Neosetophoma* showed higher % abundance ($D = 0.57$; $p < 0.05$) in 2024 ($8.25\% \pm 20.6\%$) than 2022 ($0.02\% \pm 0.05\%$) in A3-25, and *Cladosporium* showed higher % abundance ($D = 1.22$; $p < 0.01$) in 2023 ($11\% \pm 6.2\%$) than in 2024 ($3\% \pm 1.9\%$) in A35.

Of the found 4925 ASVs, < 42% were found in layers across years and < 31% were found in single years (Table 4). Considering layers as replicates, the average number of found ASVs was higher in 2024 (1138 ± 282) than in 2022 (300 ± 122; $D = 3.86$; $p < 0.01$).

Few significant year differences were found for alpha diversity (Figure S4), probably because of the huge variability of values, few observations in 2022 and the correction for multiple testing. In A0-3, observed mean ASV richness and Chao1 showed higher values ($p < 0.05$) in 2024 and 2023 than 2022 ($D_{richness}$2024–2022: 1.41; $D_{richness}$2023–2022: 0.66; D_{Chao1}2024–2023: 1.38; D_{Chao1}2024–2022: 0.68), respectively (Figure S4). In A3-25, mean observed richness and Chao1 showed higher values ($p < 0.05$) in 2024 than 2022 ($D_{richness}$2024–2022: 0.79; D_{Chao1}2024–2022: 1.11) (Figure S4). In the other layers (A35, B0, B4), no significant differences for mean observed richness and Chao1 between years were found. Mean Shannon diversity did not show any year differences in any layer (Figure S4).

Six ASVs occurred in all samples of the different layers (Table 5), irrespective of year differences. These core taxa were specific for basins (*Aspergillus versicolor* core ASV in the shallow basin—B0 and B4) or occurred across basins (*Cladosporium basi-inflatum* core ASV in A0-3, A35, B0; *Filobasidium magnum* core ASV in

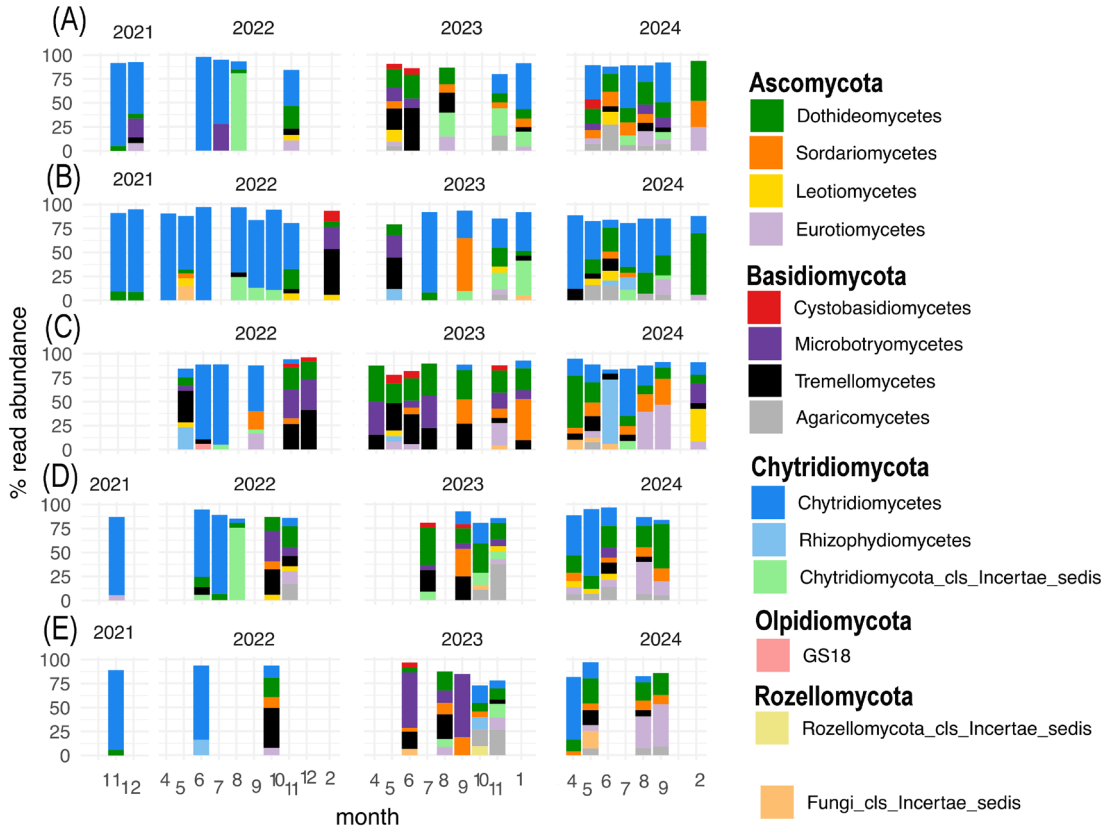

FIGURE 3 | Fungal classes with at least 4% read abundance in one sample; (A) upper euphotic layer—A0-3; (B) lower euphotic layer—A3-25; (C) deep hypolimnion—A35; (D) surface of the shallow basin—B0; (E) bottom layer of the shallow basin—B4. Numbers indicate months.

A0-3, A35 and B4). Furthermore, these core taxa generally had a low mean % abundance per sample (< 10%) but reached in certain samples also a higher % abundance (e.g., *Aspergillus versicolor* showing 39% read abundance in B4). Surprisingly, no core taxon was found for A3-25.

Focusing on ASVs that occurred in all samples of the different layers in 2022 or 2024, few unique ASVs were found in 2022 (< 2% of ASVs found) and 2024 (< 1% of ASVs found; Table 6). Remarkably, unique ASVs for 2024 were found in all layers while only in A0-3 and A35 for 2022. In 2022, ASVs belonged to four genera, while in 2024, they belonged to 13 genera.

3.4 | Differentially Abundant ASVs in Years

Because most year differences were found between 2022 and 2024, we specifically tested for differentially expressed ASVs for these years across all layers. Seventeen ASVs were differentially abundant ($p < 0.01$) that all had a mean relative abundance < 1%. Twelve ASVs (2 Ascomycota, 7 Basidiomycota, 3 Chytridiomycota) showed higher values in 2022 than 2024, and five ASVs (4 Ascomycota, 1 Basidiomycota) showed higher values in 2024 (Figure 4). Of the ASVs showing higher abundance in 2024, ASV61 (*Vishniacozyma carnescens*) exclusively occurred in 2022 in A35, while ASV26 (*Fusarium* sp.) occurred in 2024 in A35 (Table 6). ANCOM considered abundance and presence of ASVs, while the analysis about the occurrence of taxa in 2022 or 2024 only considered presence, and thus mostly different ASVs were indicated.

3.5 | Beta Diversity Indices

The mean distance of samples to their group centre was not statistically different ($p > 0.05$) for layers and years (function betadisper). Contrarily, the group centres of summer and autumn were different ($p < 0.05$).

When partitioning beta-diversity (PERMANOVA), layers were not statistically significant and only years (4.7%) and seasons (spring, summer, autumn; 6.2%) and their interaction (5.0%) explained together 15.9% of variability ($p < 0.001$). Considering the significant season effect in betadisper, mainly the year effect can be attributed to beta-diversity.

3.6 | Multivariate Ordination

In NMDS with ASVs (stress = 0.15), only the centre of autumn was different from that of the other seasons and only a slight difference in the centre of samples from A3-25 and A35 was found (Figure S5). In environmental fitting, years as factors were significantly ($R^2 = 35\%$; $p < 0.001$) related to the ordination, with samples of the years 2022 and 2024 generally separated (Figure 5).

TABLE 1 | Year differences within layers for fungal classes; year comparison (year comp.); significance level (sign.; $p < 0.05$*; $p < 0.01$**); Cohen's *D* (*D*); lower euphotic layer—A3-25; deep hypolimnion—A35. In parentheses, the phylum affiliation is shown (A, Ascomycota; B, Basidiomycota; C, Chytridiomycota).

Layer	Class (phylum)	Year comp.	Sign.; *D*	Mean % read abundance ± one standard deviation
A3-25	Spizellomycetes (C)	2022 < 2024	*; 0.87	2022 = absent 2024 = 0.04 ± 0.064
A35	Agaricomycetes (B)	2022 < 2024	*; 1.22	2022 = 0.35 ± 0.448 2024 = 2.59 ± 2.57
	Cystobasidiomycetes (B)	2024 < 2022	*; 0.62	2023 = 4.87 ± 1.88 2024 = 1.05 ± 1.04
	Fungi class incertae sedis	2022 < 2024 2023 < 2024	**; 1.71 *; 1.33	2022 = 0.18 ± 0.31 2023 = 0.80 ± 1.53 2024 = 4.27 ± 3.37
	Microbotryomycetes (B)	2024 < 2023	**; 1.61	2023 = 17.0 ± 13.2 2024 = 1.85 ± 1.28
	Rozellomycota class incertae sedis	2022 < 2024 2023 < 2024	*; 1.22 *; 1.10	2022 = 0.004 ± 0.011 2023 = 0.01 ± 0.026 2024 = 0.09 ± 0.1
	Taphrinomycetes (A)	2022 < 2024	*; 2.42	2022 = 0.007 ± 0.019 2024 = 0.144 ± 0.078

TABLE 2 | Layer differences across years for fungal classes; year comparison (year comp.); significance level (sign.; $p < 0.05$*; $p < 0.01$**); Cohen's *D* (*D*); upper euphotic layer—A0-3; lower euphotic layer—A3-25; deep hypolimnion—A35; surface of the shallow basin—B0. In parentheses, the phylum affiliation is shown (A, Ascomycota; B, Basidiomycota; C, Chytridiomycota).

Class	Layer differences	Sign.; *D*	Mean % read abundance ± one standard deviation
Chytridiomycetes (C)	A3-25 > A35	**; 1.21	A3-25: 52 ± 29.8 A35: 19 ± 25.4
Eurotiomycetes (A)	A0-3 > A3-25 A35 > A3-25 B0 > A3-25	**; 0.83 *; 0.67 *; 0.70	A0-3: 6 ± 6.5 A3-25: 2 ± 3.5 A35: 8 ± 13.3 B0: 10 ± 14.1
Microbotryomycetes (B)	A35 > A3-25	*; 0.74	A3-25: 3 ± 6.6 A35: 11 ± 12.4
Sordariomycetes (A)	A35 > A3-25	*; 0.51	A3-25: 4 ± 11.6 A35: 10 ± 11.2
Tremellomycetes (B)	A35 > A3-25	*; 0.65	A3-25: 7 ± 11.5 A35: 15 ± 12.4

TABLE 3 | Layer differences across years for fungal genera; Cohen's *D* (*D*); upper euphotic layer—A0-3; lower euphotic layer—A3-25; deep hypolimnion—A35; surface of the shallow basin—B0; $p < 0.05$*; $p < 0.01$**.

Genus	Layer comparison	Significance level; *D*	A0-3	A3-25	A35	B0
Filobasidium	A35 > A3-25 A35 > A0-3 A35 > B0	**; *D* = 0.47 **; *D* = 0.98 **; *D* = 0.93	2 ± 2.9	4 ± 7.6	7 ± 7.2	2 ± 1.8
Rhodotorula	A35 > A3-25	**; *D* = 0.75	6 ± 7.8	3 ± 3.4	10 ± 12.3	4 ± 7.8
Zygophlyctis	A3-25 > A35	*; *D* = 1.22	27 ± 30.3	40 ± 30.2	10 ± 16.4	23 ± 28.6

The pRDA explained 17.2% of ASV variability (RDA1: 10.1%; $p < 0.001$; RDA2: 4.5%; $p < 0.001$; Figure 5). Year ($p < 0.001$) and season ($p < 0.001$) as factors and pH ($p < 0.01$) and P_{tot} ($p < 0.05$) as continuous variables were related to the ordination. The effect of pH and P_{tot} was the same across layers (non-significant interaction between layer and environmental variables conditioned on year and season effects) while the year—season interaction effect was statistically significant, indicating that the seasonality was not the same in each year (Figure 5). The top 10 ASVs related to P_{tot} belonged to Ascomycota (*Cladosporium*, *Fusarium*) and Basidiomycota yeasts (*Vishniacozyma*, *Rhodotorula*) while those related to pH belonged to Chytridiomycota, mostly genus *Zygophlyctis* (Table S5). In variance partitioning, the factor layer only explained uniquely 3.4% ($p < 0.001$) of variability, contradicting our hypothesis on layer differences while environmental variables (pH, P_{tot}) explained 17.2% ($p < 0.001$) and their interaction 1.1% (Figure 6).

TABLE 4 | Richness of ASVs found in layers across years (total richness) and in single years; upper euphotic layer—A0-3; lower euphotic layer—A3-25; deep hypolimnion—A35; surface of the shallow basin—B0.

Layer	Total richness (% with respect to 4925 ASVs)	Single years		
		2022	2023	2024
A0-3	2043 (41.5%)	249	811	1497
A3-25	1734 (35.2%)	377	473	1332
A35	1470 (29.8%)	371	663	923
B0	1871 (37.9%)	393	829	1122
B4	1343 (27.3%)	108	714	814

3.7 | Network Analysis of Mycoplankton of the Deep Basin

All networks exhibited a network topology that differed statistically from that of a random null-model (configuration model). The upper euphotic layer displayed an exceptionally high value of clustering related to a rigid network composed of highly interconnected, nearly isolated modules (Table 7; Figure 7). Contrarily, the lower euphotic layer and the hypolimnion showed a lower clustering coefficient linked to more open and flexible patterns. The lower euphotic layer also had a high modularity and showed the highest centralisation index, linked to the presence of dominant hub taxa. The network diameter, average path length and the number of connections (edges) were notably higher for the lower euphotic layer than for the other layers (Table 7), linked to a highly interconnected network. The hypolimnion had the highest modularity, reflecting a highly partitioned network where taxa were organized into distinct, poorly connected groups.

ASVs of genus *Zygophlyctis* (ASV 8, 14, 16, 40; primary algal parasite) were the top hubs in all layers of the deep basin and additionally *Cystobasidium* (ASV 175; mycoparasite) in the lower euphotic layer (Figure 7). These ASVs were more abundant ($p < 0.01$) in the lower euphotic layer than the hypolimnion, except for ASV 16, that had its highest abundance ($p < 0.01$) in the lower euphotic layer than the upper euphotic layer. In the lower euphotic layer, *Zygophlyctis* was connected to *Cystobasidium*, while in the other layers, no such linkage was found.

3.8 | Path Analysis

In path analysis, variability explained and relationships found were essentially not different for observed richness and

TABLE 5 | Fungal ASVs specific for layers occurring in all samples (core ASVs); taxonomic levels were truncated at phylum level (mycota—m.), class level (mycetes—m), order level (ales—a.) and family level (ceae—c.); upper euphotic layer—A0-3; lower euphotic layer—A3-25; deep hypolimnion—A35; surface of the shallow basin—B0. Bold, italicized or underlined ASV numbers indicate that these ASVs were common in several layers; range indicates the % read abundance across all samples of the respective layer.

Layer	ASV	Phylum	Class	Order	Family	Genus	Species	Range
A0-3	**13**	Basidiom.	Tremellom.	Filobasidia.	Filobasidiac.	*Filobasidium*	*magnum*	0.01%–10%
	22	Ascom.	Dothideom.	Capnodia.	Cladosporiac.	*Cladosporium*	*basi-inflatum*	0.03%–5%
	59						*herbarum*	0.01%–2%
A35	**13**	Basidiom.	Tremellom.	Filobasidia.	Filobasidiac.	*Filobasidium*	*magnum*	0.3%–24%
	18		Microbotryom.	Sporidiobola.	Sporidiobolac.	*Rhodotorula*	*mucilaginosa*	0.04%–17%
	22	Ascom.	Dothideom.	Capnodia.	Cladosporiac.	*Cladosporium*	*basi-inflatum*	0.05%–7%
B0	*11*	Ascom.	Eurotiom.	Eurotia.	Aspergillac.	*Aspergillus*	*versicolor*	0.1%–30%
	22		Dothideom.	Capnodia.	Cladosporiac.	*Cladosporium*	*basi-inflatum*	0.4%–12%
	43			Pleospora.	Pleosporac.	*Alternaria*	sp.	0.08%–4%
B4	*11*	Ascom.	Eurotiom.	Eurotia	Aspergillac.	*Aspergillus*	*versicolor*	0.07%–39%
	13	Basidiom.	Tremellom.	Filobasidia	Filobasidiac.	*Filobasidium*	*magnum*	0.09%–37%

TABLE 6 | Layer-specific ASVs that occurred in all samples of 2022 but not of 2024 (specific 2022) or occurred in all samples of 2024 but not of 2022 (specific 2024); taxonomic levels were truncated at phylum level (mycota—m.), class level (mycetes—m), order level (ales—a.) and family level (ceae—c.); upper euphotic layer—A0-3; lower euphotic layer—A3-25; deep hypolimnion—A35; surface of the shallow basin—B0. Empty cells indicate no assignment at that taxonomical level. Column ASV reports the ASV identity as universal identifier. In bold, ASVs that were differentially abundant in years as indicated by ANCOM.

Year	Layer	ASV	Phylum	Class	Order	Family	Genus	Species
Specific 2022	A0-3	49	Chytridiom.	Chytridiom.	Rhizophydia.			
		104			Zygophlyctida.	Zygophlyctidac.	*Zygophlyctis*	sp.
	A35	**61**	Basidiom.	Tremellom.	Tremella.	Bulleribasidiac.	*Vishniacozyma*	*carnescens*
		164			Filobasidia.	Filobasidiac.	*Filobasidium*	*wieringae*
		236	Chytridiom.					
		312						
		325	Ascom.	Sordariom.	Hypocrea.	Nectriac.	*Neonectria*	sp.
Specific 2024	A0-3	42	Ascom.	Dothideom.	Capnodia.	Cladosporiac.	*Cladosporium*	*halotolerans*
		57						*basi-inflatum*
		58	Basidiom.	Microbotryom.	Sporidiobola.	Sporidiobolaceae	*Rhodotorula*	sp.
	A3-25	57	Ascom.	Dothideom.	Capnodia.	Cladosporiaceae	*Cladosporium*	*basi-inflatum*
	A35	19	Ascom.	Dothideom.	Pleospora.	Phaeosphaeriaceae	*Neosetophoma*	*samarorum*
		26		Sordariom.	Hypocrea.	Nectriaceae	*Fusarium*	sp.
		60		Eurotiom.	Chaetothyria.	Herpotrichiellaceae	*Exophiala*	*xenobiotica*
		142				Cyphellophoraceae	*Cyphellophora*	*europaea*
		192			Eurotiales	Aspergillaceae	*Penicillium*	*chrysogenum*
	B0	170	Basidiom.	Agaricom.	Agaricales	Schizophyllaceae	*Schizophyllum*	*commune*
		213		Malasseziom.	Malasseziales	Malasseziaceae	*Malassezia*	*restricta*
		228		Agaricom.	Polyporales	Polyporaceae	*Trametes*	*versicolor*
		308				Phanerochaetaceae	*Bjerkandera*	sp.
	B4	57	Ascom.	Dothideom.	Pleosporales	Phaeosphaeriaceae	*Dematiopleospora*	sp.
		139	Basidiom.	Agaricom.	Russulales	Bondarzewiaceae	*Heterobasidion*	*occidentale*

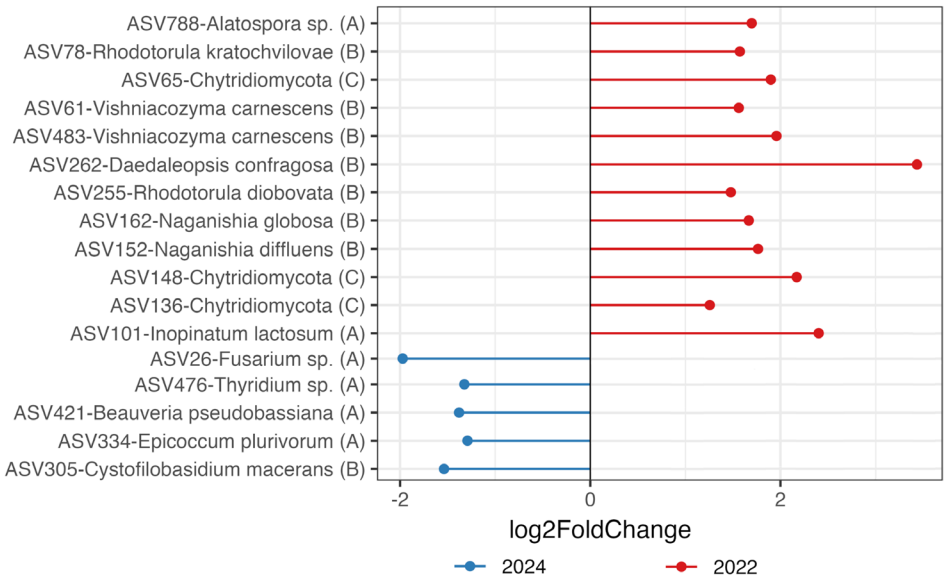

FIGURE 4 | Seventeen differentially abundant ASVs in the years 2022 and 2024 as indicated by ANCOM: red indicates increase in 2022, blue in 2024. All ASVs had a mean % read abundance < 1%; Ascomycota (A), Basidiomycota (B), Chytridiomycota (C).

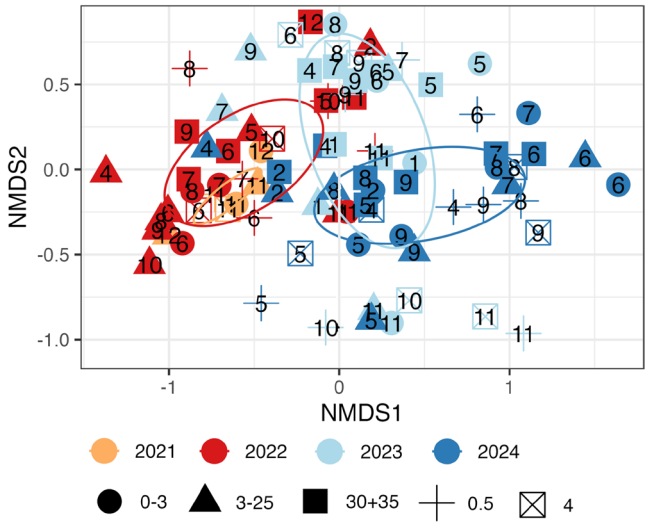

FIGURE 5 | NMDS (stress = 0.16) with fungal ASVs and environmental fitting of years (95% envelope of confidence interval for year centre; $p < 0.001$); upper euphotic layer—A0-3; lower euphotic layer—A3-25; deep hypolimnion—A35; surface of the shallow basin—B0; bottom layer of the shallow basin—B4. Numbers indicate months.

Chao1 diversity ($R^2_{\text{observed richness}}$: 24%, $R^2_{\text{Chao1 diversity}}$: 23.7%). Precipitation and % DO directly increased and pH directly decreased fungal diversity (Table 8). Air temperature did not influence fungal diversity. Silica and % DO and pH, respectively, were negatively correlated. Precipitation significantly reduced silica that was not a predictor of fungal diversity. No significant indirect pathways were found for precipitation.

4 | Discussion

We investigated the diversity of fungal communities in different layers and years of mountain Lake Tovel. In general, the fungal phyla dominating in Lake Tovel also dominate in different aquatic habitats (lakes, ice and snow, polar systems, coasts: Grossart et al. 2019; Scandinavian lakes along a longitudinal gradient: Khomich et al. 2017; hypersaline lakes: Mircea et al. 2024). The dominant fungal genera in Lake Tovel occur across different habitats (*Fusarium*: deep sea, aquifer, low-land bathing lakes; Grossart et al. 2019; Nowacka et al. 2018; *Cladosporium*, *Aspergillus*, *Filobasidium*, *Rhodotorula*, *Fusarium*: boreal and subarctic lakes: Sanyal et al. 2025; *Alternaria*: Tibetan Plateau lakes; Phurbu et al. 2025; *Aspergillus*: low-land, oligotrophic Lake Ohrid; Čomić et al. 2010). Furthermore, dominant fungal phyla, orders and genera occur consistently across boreal and subarctic lakes (Sanyal et al. 2025). Also here, dominant genera found in Lake Tovel were found in different layers. Thus, fungal taxa found in Lake Tovel occur across large environmental gradients spanning different trophic states, climate and latitude, and we suggest that a large-scale study across these gradients is needed to infer the uniqueness of the fungal diversity of mountain lakes.

The core fungal community of Lake Tovel occurring in all layer-specific samples consisted of only six ASVs that rarely showed high abundance. Rare species are increasingly recognized as an important component of ecosystems as they provide key functions in biogeochemical cycles (Jousset et al. 2017). Among the core taxa, *Filobasidium magnum* is found in subglacial ice (Perini et al. 2019), *Rhodotorula mucilaginosa* produces carotenoids and triacylglycerol lipids (Li et al. 2022), important compounds for UV protection and cold adaptation, *Rhodotorula mucilaginosa* and *Cladosporium herbarum* occur in five mesotrophic lakes of Poland (Pietryczuk et al. 2025), *Aspergillus* species are common in terrestrial, freshwater and marine environments (Wurzbacher et al. 2016) and specifically *A. versicolor* produces a potent antibiotic (King et al. 2014). We suggest that these core taxa showed a vast adaptation in Lake Tovel given their year-round presence, driven by aspects such as cold adaptation and success in interspecies interactions.

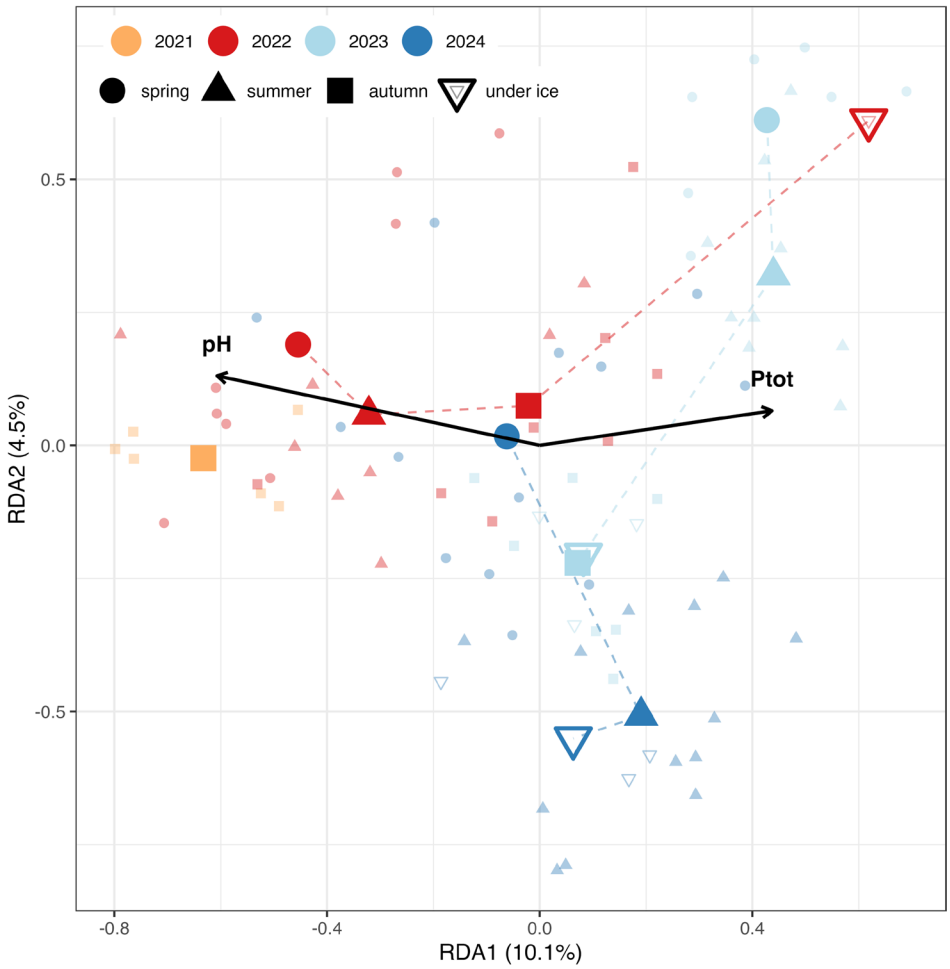

FIGURE 6 | Partial redundancy analysis of fungal communities, controlled for layer-specific effects. Dashed lines indicate the seasonal succession within each year. Symbols relate to seasons and colours to years.

TABLE 7 | Structural network characteristics of layers of the deep basin (upper euphotic layer—A0-3; lower euphotic layer—A3-25; hypolimnion—A30-35).

	A0-3	A3-25	A30-35
Vertex	37.00	49.00	44.00
Edge	42.00	73.00	40.00
Average degree	2.27	2.98	1.82
Average path length	1.04	2.30	1.22
Network diameter	3.00	7.00	3.00
Clustering coefficient	0.96	0.75	0.75
Density	0.06	0.06	0.04
Heterogeneity	0.84	0.74	0.62
Centralization	0.10	0.13	0.05
Modularity	0.70	0.71	0.86

Our study period covered 2 years with different weather patterns. As in different areas in Northern Italy (Copernicus Climate Change Service and World Meteorological Organization 2025), the year 2024 was very rainy at Lake Tovel. In Lake Tovel, cold spells and heavy rain induce deep-water oxygenation of the hypolimnion, a process not indicated by mixing indices (Flaim et al. 2020). Accordingly, the higher hypolimnetic % DO in 2024 than in other years can be attributed to deep-water oxygenation by rainfall-induced water column mixing, both in rainy November 2023 and in rainy year 2024. The year 2022 was one of the hottest in Europe (Ballester et al. 2023). Accordingly, summer air temperatures were higher in 2022 than in other years at Lake Tovel. Air temperature and water temperature are tightly connected (Edinger et al. 1968), explaining warmer water temperatures in different layers in 2022 compared to 2024. The temperature rise in the hypolimnion in August and October 2024 can be attributed to earlier mixing bringing warm surface water to the deeper layer according to Flaim et al. (2020). Changes in lake temperature and hydrology can affect key biological processes by nonlinear interactions (Adrian et al. 2009). Among environmental variables, pH was higher in all layers in 2022 than in 2024. This pH increase can be related to many factors such as intensified photosynthesis, carbonate dissolution and organic matter uptake (Castrillon-Munoz et al. 2022). Without hypothesising on the underlying causes, this observation also indicated substantial environmental differences between the warm 2022 and rainy 2024 that potentially influenced fungal communities.

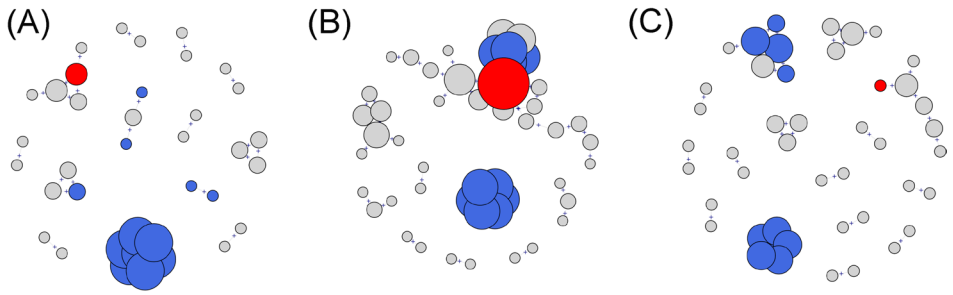

FIGURE 7 | Network of fungal taxa at the deep basin. Blue nodes represent genus *Zygophlyctis* (primary algal parasite) and red nodes represent genus *Cystobasidium* (mycoparasite). Node size is proportional to the number of connections (degree). (A) The network of the upper euphotic layer is characterized by a rigid structure with highly interconnected but isolated hubs; (B) The network of the lower euphotic layer is a highly integrated network centred around two hubs; (C) The network of the hypolimnion is fragmented and weakly connected with reduced hub importance (degree < 5).

TABLE 8 | Summary results of path analysis for observed fungal diversity (fungal div). Standardized coefficients and significance levels ($p < 0.05$*; $p < 0.001$***) are reported.

Path type	Relationship	Coefficient	*p*
Direct	Precipitation → fungal div	0.3	***
	% DO → fungal div	0.4	*
	pH → fungal div	−0.4	***

Alpha diversity indices (richness and Chao1) were higher in 2024 than 2022 in the upper and lower euphotic layer. The % abundance of Basidiomycota and of the most abundant genera in the lower euphotic layer and the % abundance of most fungal classes in the lower euphotic layer and hypolimnion were higher in 2024 than 2022. Thus, there was a tendency to have higher diversity in 2024. Path analysis indicated that increased precipitation increased fungal diversity. The importance of transport by rain and wind for fungi into surface waters is known (Sherry 1986; Khomich et al. 2017), but it is difficult to distinguish between indwellers or transported fungi, especially with eDNA studies (Sanyal et al. 2025). Based on functional traits, Li et al. (2025) show that rainfall decreases the abundance of fully aquatic fungi and increases the abundance of partially aquatic fungi. We suggest that the higher diversity of fungal communities in rainy 2024 can be attributed to different aspects such as wash-in of fungi and of allochthonous material by rain. Also in rivers, Basidiomycota dominate during the rainy season, attributed partially to terrestrial run-off (Siriarchawatana et al. 2024).

In NMDS and pRDA, a clustering of samples from 2022 and 2024 was found. Specifically, in the pRDA, mostly Chytridiomycota of the genus *Zygophlyctis* were positively linked to high pH (8.1 ± 0.37) in 2021 and 2022 while Ascomycota (*Cladosporium*, *Fusarium*) and Basidiomycota yeasts (*Vishniacozyma*, *Rhodotorula*, *Cystobasidium*) were positively linked to high P_{tot} in 2023 and 2024. Path analysis indicated that increasing pH decreased fungal diversity. This might indicate that the effect of decreased diversity was more pronounced for Ascomycota and Basidiomycota. In fact, Chytridiomycota show acidophilic and alkalophilic ecotypes adapted to a wide range of pH (Gleason et al. 2010). pH is an important driver of fungal diversity and community composition in freshwater habitats (Ortiz-Vera et al. 2018; Wurzbacher et al. 2016) but pH does not influence mycoplankton richness of over 300 Canadian lakes (Gauthier et al. 2025). We argue that in large-scale analyses often idiosyncratic patterns disappear. In a coastal watershed, functional diversity of aquatic fungi increases with total particulate phosphorus, linked to the richness of algal parasites. Because Lake Tovel is oligotrophic (Cellamare et al. 2016), we continue this line of reasoning and hypothesise that high phosphorus led to algal growth inducing proliferation of chytrids that themselves were preyed on by *Cystobasidium*. *Cladosporium* and *Fusarium* are saprotrophs, and their increase could be linked to wash-in of litter in rainy 2024. While only experiments can clarify the underlying relationships, our results indicated a separation of mycoplankton based on water chemistry and ultimately years. Thus, according to our first hypothesis, the fungal community of Lake Tovel was different between the years 2022 and 2024 but the differences were not noted in all layers and not consistently for layers. Missing differences can be related to different causes such as the lower sequencing success for samples of the bottom of the shallow basin (B4), unresponsive communities and not all metrics are equally sensible to environmental changes (Santini et al. 2017).

Myco- and phytoplankton are interlinked by processes such as assimilation and decomposition of phytoplankton derived organic matter (Cunliffe et al. 2017), influence on phytoplankton population dynamics (Sassenhagen et al. 2023), and trophic linkage between phyto- and zooplankton (Kagami et al. 2014). In Lake Tovel, the phytoplankton is considered cold-water adapted (Cellamare et al. 2016). There was a tendency to find chl-a maxima at deeper depths in 2022 than 2024, probably induced by warmer water temperatures in 2022. We suggest that changes in the spatial distribution of the chl-a maximum as a proxy for phytoplankton biomass and thus algal communities also influenced fungal community differences between years. We acknowledge that the occurrence of deep chlorophyll maxima is influenced by many factors such as light availability, the vertical distribution of nutrients, the location of thermal gradients and bio-acclimation (Leach et al. 2018), but this result adds up to the many evidences of year differences.

Also, several ASVs were differentially abundant in 2022 and 2024. Among the ASVs showing higher abundance in 2022, *Vishniacozyma* species are mainly endophytes, epiphytes and

saprophytes of plants, especially of leaves (Liu et al. 2025) and are found in the litter and soil of temperate beech, oak and spruce forests (Tomšovsky et al. 2017). *Rhodutorula* is a patho-saprotroph fungus, *Daedaleopsis confragosa* is a wood decaying fungus (Põlme et al. 2020). Among the ASVs showing higher abundance in 2024, *Beauveria* is an animal pathogen, *Epicoccum* and *Fusarium* are plant pathogens and wood saprotrophs (Põlme et al. 2020). Projected warming by climate change will likely accelerate wood decomposition and significantly decrease the residence time in decay stages (Chagnon et al. 2022). The dry and warm year 2022 negatively affected deciduous broadleaf trees in frests across Europe (Gharun et al. 2024). Trees are standing close to the shore at Lake Tovel. We hypothesise that the higher abundance of leaf associated and wood decaying fungi in 2022 was linked to heat stress of trees while in 2024 we attribute their presence to wash-in effects. We acknowledge that this hypothesis is speculative, but the observed differences clearly indicated the influence of different years on mycoplankton, even though the underlying causes remain challenging to identify. While there was a clear indication of year differences, season differences in fungal communities were weak in Lake Tovel, similar to Lake Mekkojärvi (Sweden; Sanyal et al. 2025).

Layer differences were mostly found for phyla, classes, and genera between the intermediate layer and the hypolimnion and for network characteristics of layers of the deep basin but not for alpha diversity and not in NMDS, pRDA and variance partitioning. This indicated that only the consideration of diverse aspects provided a complete and multifaceted picture of diversity. Only three of the ten most abundant genera (*Rhodotorula* and *Filobasidium* higher abundance in the hypolimnion than the lower euphotic layer; *Zygophlyctis* and Chytridiomycetes in general higher abundance in the lower euphotic layer than the hypolimnion) showed layer differences. The genus *Rhodotorula* includes ubiquitous saprotrophic yeasts isolated from different aquatic habitats and invertebrates (Nagahama et al. 2003) and is widely found in the epilimnion (Khomich et al. 2017) and in the marine oxygen minimum zone (Peng and Valentine 2021; Buedenbender et al. 2020). *Filobasidium* is associated with low % DO in subtropical high-altitude wetlands (Shen et al. 2025). *Zygophlyctis* is a parasite on diatoms and decreases the diatom sinking rate by preventing aggregate formation (Klawonn et al. 2023). Little is known about the distribution of microbial interactions across the water column (Deutschmann et al. 2024). Here, the upper euphotic layer was characterized by highly clustered, isolated modules indicating a high local specialisation. The upper euphotic layer is UV impacted (Obertegger et al. 2008) and most exposed to seasonal forcing. The lower euphotic layer showed the highest topological complexity, indicative of robustness against perturbations (Cornell et al. 2023; Ma et al. 2025). The interaction between *Zygophlyctis* (primary algal parasite) and *Cystobasidium* (mycoparasite) pointed to hyperparasitism in the lower euphotic layer. Hyperparasitism, parasites infecting other parasites, is likely an ecosystem stabilizing force and very common in nature (Parratt and Laine 2016). The hypolimnion showed the highest modularity, possibly as protection against perturbations in this low temperature and low oxygen system. Several genera such as *Cladosporium*, *Penicillium* or *Neocucurbitaria* were specific to the hypolimnion. These genera are deep sea fungi that possess unique physical and biochemical mechanisms to cope with anoxic, dark and low-temperature environments (Wang et al. 2020). Thus, contrarily to our second hypothesis, layer differences in fungal communities were not as marked as for bacterial communities in Lake Tovel (Obertegger et al. 2018) and were mostly linked to specific phyla and genera, and also statistical analyses. Mycoplankton communities often show spatial uniformity across different zones in oligo-mesotrophic lakes (Monchy et al. 2011; Wurzbacher et al. 2016) while eutrophic (Cudowski and Pietryczuk 2020), and boreal and subarctic (Sanyal et al. 2025) lakes exhibit clear differences in fungal communities between upper and lower water layers. Also, network metrics of fungal communities change along depth (Deutschmann et al. 2024). Fungi show morphological changes (Song and Kumar 2012), adjust their growth direction (Yu and Fischer 2019), alternate between different reproductive strategies (Jung et al. 2014) and change their metabolic pathways in response to changes in substrate and environmental conditions (Fox and Howlett 2008). This high adaptability in relationship with environmental conditions might explain the different patterns found at Lake Tovel and across other lake studies.

Monthly sampling in general and our restricted sampling in certain layers might have missed and obscured small-scale changes in fungal communities. Furthermore, the non-consideration of the origin of fungi might have smoothened layer, season and/or year differences. Despite these shortcomings, we found year differences, and our study provided insights into monthly fungal community dynamics of a mountain lake, an understudied habitat (de Souza, Convey, et al. 2022). While it is known that fungal communities have the potential as bioindicators for water quality (Cudowski and Pietryczuk 2020; Fang et al. 2025), we showed that fungal communities can also indicate environmental changes linked to altered weather patterns, an important aspect considering that with climate change precipitation patterns are changing in mountain areas (Pepin et al. 2022). Here, layer differences were less marked than expected, linked to specific fungal taxa and methods used.

Author Contributions

Conceptualisation: U.O. Data analysis: U.O., S.C., L.C., M.P. Preparation of figures and tables: U.O., S.C., L.C., M.P. Conducting the research, data interpretation, writing: U.O., S.C., L.C., M.P.

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Conflicts of Interest

Obertegger Ulrike is an editor of FWB and co-author of this article. She was excluded from editorial decision-making related to the acceptance of this article for publication in the journal. Other co-authors have declared that no conflicts of interest exist.

Data Availability Statement

Raw ITS sequences have been deposited to the European Nucleotide Archive (ENA) with study accession number PRJEB98722. Environmental data are available from the corresponding author on reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Fluoroprobe profiles from different months during different years taken over the deepest basin. Numbers in panels indicate months. **Figure S2:** Rarefaction curves for samples from different layers. The threshold for rarefying samples (read abundance = 10,179) for alpha diversity analyses is indicated by the black vertical line. Upper euphotic layer—A0-3; lower euphotic layer—A3-25;

deep hypolimnion—A35; surface of the shallow basin—B0; bottom of the shallow basin—B4. **Figure S3:** % Read abundance of phyla in different layers; (A) upper euphotic layer—A0-3; (B) lower euphotic layer—A3-25; (C) deep hypolimnion—A35; (D) surface of the shallow basin—B0; (E) bottom layer of the shallow basin—B4. Aphelidiomycota, Blastocladiomycota and Mucoromycota are not shown because having < 1% read abundance in samples. Numbers indicate months. **Figure S4:** Boxplot of alpha diversity indices (observed richness—observed; Chao1 diversity—Chao 1; Shannon Wiener diversity—Shannon) for layers and years; upper euphotic layer—A0-3; lower euphotic layer—A3-25; deep hypolimnion—A35; surface of the shallow basin—B0; bottom layer of the shallow basin—B4. Points indicate observed values. **Figure S5:** NMDS (stress = 0.16) with fungal ASVs and 95% confidence envelopes for seasons (upper panel: spring—sp.; summer—su; autumn—au; under ice—ice) and layers (lower panel: upper euphotic layer—0–3; lower euphotic layer—3–25; deep hypolimnion—30 + 35; surface of the shallow basin—0.5; bottom of the shallow basin—4). **Table S1:** Temporal and spatial distribution of samples with successful ITS sequencing. X indicates a sample; deep basin (A), shallow basin (B). Colours indicate the grouping of monthly samples to years. **Table S2:** Overview of heat (red) and cold (blue) spells based on a 12-year period (2014–2025; day/month/year). **Table S3:** Year comparisons between pH in the different layers; Cohen's *D* (*D*). **Table S4:** Overview of the top 10 most abundant genera in different layers of Lake Tovel (upper euphotic layer—A0-3; lower euphotic layer—A3-25; deep hypolimnion—A35; surface of the shallow basin—B0; bottom of the shallow basin—B4). The yellow cell indicates genera that are exclusively most abundant in a single layer; bold taxa names indicate genera that are most abundant in all layers. **Table S5:** Top 10 ASVs with their taxonomy that showed a high affinity (highest scores) to P_{tot} or pH in pRDA. Bold indicates differentially abundant ASVs or year specific ASVs. **Data S1:** R-script for setting the minimal model in path analysis including only significant relationships using package lavaan.