SEASONAL TRENDS IN MYCOPLANKTON DIVERSITY AND FUNCTION IN LAKE BONNEY OF THE MCMURDO DRY VALLEYS, ANTARCTICA

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ABSTRACT

Interactions between phytoplankton and the microbial loop impact carbon fluxes within aquatic ecosystems. Some aquatic fungi represent phytoplankton parasites and drive a parasite-mediated pathway known as the mycoloop, which allows for enhanced access to phytoplankton resources. This is important in systems which rely heavily on phytoplankton for carbon, such as the polar lakes of the McMurdo Dry Valleys (MDV). The polar winter is a poorly studied season in the MDVs. Further, little is known about the contribution of fungi to the MDV microbe-dominated ecosystem, much less their seasonal patterns. Here, we explore how the diversity and activity of fungi changes across seasonal scales and the impacts that changes in fungal activity may have on the ecosystem. We found that fungal communities in Lake Bonney are dominated by parasitic Chytridiomycota and Rozellomycota, which display higher abundance in the winter. Co-occurrence networks revealed winter communities are smaller and better connected, with Rozellomycota & Chytridiomycota enhancing connectivity in the winter. Together, this suggests higher activity of the mycoloop in the winter. The co-occurrence networks also identified potential predator/prey associations which differ seasonally. Our work suggests that the mycoloop is an important part of the MDV lake ecosystems, displaying complex interactions which differ seasonally.

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DEDICATION

Para Opa.
Tu manera de ser era como un ogro,
pero nos amabas con tus formas raras.

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1) Introduction

The Microbial Loop and aquatic Carbon Cycling

Aquatic microbes, chiefly photosynthetic bacteria and protists, form a critical part aquatic food webs. because they fill the critical role as the main source of newly synthesized organic carbon into these systems (Nianzhi Jiao et al. 2024); (Johnson and Bif 2021). By converting light energy into sugars, proteins, and other organic molecules, this energy then reaches higher trophic levels through grazing by zooplankton. Within marine ecosystems, phytoplankton are estimated to fix a net ~53 Pg C m-2 yr-1 (Johnson and Bif 2021; Silsbe et al. 2016), though not all of this reaches higher trophic levels. About 20% of this carbon is exported via the biological carbon pump (BCP) (Palmer and Totterdell 2001) to the ocean depths. Through the sinking of larger algae, fecal pellets, or phytoplankton aggregates, carbon is exported from the photic zone to the ocean depths where they can remain sequestered for upwards of 100 years (Nianzhi Jiao et al. 2024). Recent work has also considered the importance of this carbon pump within lake systems. Lakes are now considered significant components of carbon cycling and can vary from carbon sink to source depending on food web structure (Tranvik, Cole, and Prairie 2018). These food web structures, and the biological carbon pump, are regulated by physical, chemical, and biological feedback loops. These biological feedback loops are dependent on the interactions between phytoplankton and their zooplankton grazers and the present phytoplankton species (Irion et al. 2021). Beyond grazing impacts from zooplankton, interactions within the microbial community, or Microbial Loop, can impact the efficiency of the biological carbon pump (Nianzhi Jiao et al. 2024).

The Microbial Loop is defined as the pathway by which, through microbial activity, dissolved organic carbon (DOC) is returned to higher trophic levels (Nianzhi Jiao et al. 2024). The basic microbial loop model consists of phytoplankton, bacteria, and predatory protists. Phytoplankton, such as cyanobacteria and green algae, fix CO_2 through photosynthesis (autochthonous carbon), some of which is released by phytoplankton in the form of DOC. This DOC can be taken up by bacteria. Further, DOC coming in from streams (allochthonous carbon) acts as a carbon subsidy and can enhance bacterial growth. Predatory protists such as heterotrophic nanoflagellates (HNF's) are then able to consume these bacteria. These can then be predated on by larger protist predators such as ciliates or zooplankton (Nianzhi Jiao et al. 2024).

Since its recognition, the microbial loop has been the focus in understanding aquatic carbon cycling in oceans (Nianzhi Jiao et al. 2024; N. Jiao et al. 2014) and, more recently, in lake ecosystems (Tranvik, Cole, and Prairie 2018). An important facet of this is understanding how the interactions between phytoplankton, bacteria, and larger predators impacts the efficiency of lakes and oceans to sequester carbon (Nianzhi Jiao et al. 2024; Tranvik, Cole, and Prairie 2018; N. Jiao et al. 2014). However, these studies neglect the role that aquatic fungi have within aquatic systems.

Fungi and the Mycoloop

Fungi are a morphologically and metabolically diverse protist group belonging to the Subdivision Opisthokonta. This metabolic diversity has allowed them to spread to almost every biome and allows them to take advantage of various energy rich carbon molecules such as lignin, xylan, and chitin to name a few (Sampaio et al. 1999; Gałązka et al. 2022; Naranjo-Ortiz and Gabaldón 2019). Fungi largely exist as saprotrophs, symbionts, or parasites of algae, amoeba, and even other fungi (Naranjo-Ortiz and Gabaldón 2019). Terrestrial fungal species are also well studied due to the impacts they have on nutrient cycling within soils, the mycorrhizae of many trees, and due to their abundance in soil. However, it is thought that a vast majority of fungi in aquatic systems remain undiscovered: a thought that was propelled by the increasing amount of biological ‘dark matter’ found in environmental DNA samples (Grossart et al. 2016). Biological dark matter refers to microbes known only by their genetic data within environmental samples: a grand part of these likely belonging to fungi and many falling disproportionately into the recently characterized basal lineages of Rozellomycota (Cryptomycota), Chytridiomycota, and Aphelidiomycota (Letcher and Powell 2018; 2019; Kaczmarek and Boguś 2021). These basal lineage fungi are characterized by a largely parasitic lifestyle and either flagellated or amoeboid zooflagellate stages (Kaczmarek and Boguś 2021; Letcher and Powell 2018; 2019; Kagami, Miki, and Takimoto 2014). These three groups are capable of parasitizing algae, though Aphelidiomycota shows a higher specificity towards green algae (Letcher and Powell 2019). When attached to a host, these fungi will feed, grow, and reproduce within the host. Eventually this leads to host death and the release of zoospores (Kaczmarek and Boguś 2021; Kagami, Miki, and Takimoto 2014). These pathotrophs can utilize/remineralize normally inedible carbon sources, such as sinking algae, and return it to the microbial loop. The activity of these fungal

pathotrophs within the microbial loop is known as the mycoloop (Rasconi, Niquil, and Sime-Ngando 2012; Kagami, Miki, and Takimoto 2014; Grossart et al. 2019; M. Chen, Gao, and Zhang 2024), with these groups of fungi referred to as mycoplankton. The mycoloop represents the flow of carbon from phytoplankton via parasitism which can bypass the microbial loop. Further, this makes phytoplankton derived material more accessible to other predatory protists.

The impact of fungi in aquatic systems has been historically underappreciated, with the mycoloop representing a more recent (within the past decade) discovery (Rasconi, Niquil, and Sime-Ngando 2012; Thongthaisong et al. 2022). These pathotrophs are capable of directly interacting with phytoplankton such as green algae and as such may be having large consequences on the microbial loop. As algae get infected with mycoplankton, carbon and other nutrients are pulled away from the algae into the developing sporangia. Eventually, these release zoospores which are rich in fats which make them ideal prey for predatory protists. In tandem with leaking DOC from the algae, which changes bacterial interactions with algae, nutrients and carbon are recycled more quickly through the loop as infection progresses. One study focusing on Chytridiomycota (Chytrids) found that this shunt can be quite significant, funneling around 20% of photosynthetically fixed carbon from a diatom community at 50% infection to the zoospores and surrounding bacterial community via leaking host cells (Klawonn et al. 2021). Though less is known about the impacts of Aphelidiomycota (Aphelids) and Rozellomycota (Rozellids) to carbon flow, it can be expected that these parasites also change the activity of the MCP either by reducing grazing pressure on phytoplankton by producing more nutritious prey, releasing nutrients for phytoplankton to use, or shifting bacteria-algae interactions (Kagami, Miki, and Takimoto 2014; Ilicic and Grossart 2022). Additionally, saprotrophic fungi which are also reliant on photosynthetic carbon can impact RDOC via decomposition. Aggregation of saprotrophs on particles can also increase transport via the BCP, serving as a competing flux to the mycoloop (Grossart et al. 2019; Ilicic and Grossart 2022). The competition between the mycoloop and this mycoflux remains a large question (Ilicic and Grossart 2022). Recent work has shown that these mycoplankton show strong seasonal patterns related to the abundance of algal prey or highly available POC (Van den Wyngaert et al. 2022). However, there is still limited research into how the seasonality of these mycoplankton impacts the rest of the microbial loop. Well established systems with simple food webs would be ideal to address these questions. One such environment is the McMurdo Dry Valley lakes.

The McMurdo Dry Valleys

Located within the Transantarctic Mountain range, the McMurdo Dry Valleys (MDV) represents one of the largest ice-free regions in Antarctica due to both the low humidity and the high mountains preventing iceflow from the East Antarctic ice sheet (Green and Lyons 2009). The region has a diverse geology, ranging from dunes & wind carved stones to permanently ice-covered lakes. These environments are teeming with microbial life, with the largest organisms being nematodes found within soils (Fountain et al. 2014). These ecosystems have been well studied over the 30 years since the founding of the McMurdo Long Term Ecological Research project (McMLTER), with Lake Bonney, Lake Hoare, and Lake Fryxell housing permanent camps within the Taylor Valley. These lakes are perennially ice-covered with waters ranging from brackish to hypersaline. This permanent ice cover limits wind driven mixing, meaning that these lakes are also permanently stratified and harbor unique microbial communities which differ by depth. Due to this stratification, these lakes also have sharp chemical gradients which further drive community differences. The past 30 years of work has revealed diverse protist communities, with recent studies (Rojas-Jimenez et al. 2017; Marchetta et al. 2023) revealing high abundances of Rozellomycota and Chytridiomycota. These recent studies identified primer sets more effective in capturing the diversity of cryptic fungi (Rojas-Jimenez et al. 2017; Marchetta et al. 2023; Tedersoo et al. 2015). This makes these lakes an ideal place to study how these mycoplankton associate and interact with their algal hosts. Though not exact analogs to common marine or temperate lake systems, the simplicity of these lake ecosystems, the diversity of biological habitats, and the limited human disturbance make them ideal to study microbial interactions while reducing confounding variables from larger organisms (Green and Lyons 2009). Further, these polar lakes experience extreme seasonality due to the complete loss of light during the polar winter.

The Polar Night

The dynamics between polar day and polar night have been of increasing interest in the Dry Valley lakes. These lakes experience drastic seasonal shifts with summer lasting from November Through February (4 months) and winter from April to September (6 months) with only about a month for autumn in March and a month for spring in October (Obryk et al. 2020).

Polar night research has been logistically limited in these lakes, resulting in limited chemical and physical data. Biological studies during this period have been lacking, largely due to the assumption that communities simply enter dormancy during these periods. However, recent work has shown that that is not the case (Vick-Majors, Priscu, and A Amaral-Zettler 2014; Wietz et al. 2021). Winter microbial communities show rich bacterial communities with higher rates of bacterial respiration than expected. To further explore these communities during the polar night, a suite of autonomous lake profile samplers (ALPS) were installed in Lake Bonney during the 2013 field season. The ALPS project collected valuable data as to the seasonal shifts in lake conditions and the microbial community assemblages (Winslow et al. 2014). This allowed for insights into how the communities are surviving the polar night (Patriarche et al. 2021). For example, fluoroprobe data, which measures chlorophyll-a (chla) and serves as a proxy of algal abundance, shows increases chlorophyl for mixotrophic algae, suggesting that communities remain active and accrue biomass during the polar night (Vick-Majors, Priscu, and A Amaral-Zettler 2014; Patriarche et al. 2021). Other biological data collected included environmental DNA samples (eDNA) which were collected and sequenced to capture how the bacterial and protist communities were shifting across seasons. However, the chosen primer set to explore the protist community, which covers the V9 region of the 18S rRNA, did not capture the diversity of basal lineage fungi characterized by Rojas-Jimenez et al. To that end, the goal of this study was to address unanswered questions on how the MDV aquatic fungal communities respond to seasonal variation, as well as the implications this has on the mycoloop in these systems.

Thesis Objectives

To explore this question, we selected archived ALPS eDNA samples from the 2015-2016 deployment to sequence with two primers sets tuned towards fungi. These primers targeted the ITS2 region, a hypervariable region which falls between the two rRNA subunits in protists, and the V7-V8 region of the 18S rRNA which are more specific to basal fungal lineages such as Chytridiomycota and Rozellomycota (Rojas-Jimenez et al. 2017; Tedersoo et al. 2015). This was done with the overarching goal of discerning seasonal patterns in the abundance of these taxa and beginning to understand how the mycoloop fits into the McMurdo Dry Valley lake ecosystems. The goals of this study are to 1) characterize the fungal diversity present in Lake Bonney in the MDV which may have been missed by the V9 primer set, 2) characterize how the

protist communities identified by both primer sets shifted between polar day and night and explore which taxa define each season, and 3) using a co-occurrence network approach, assess how mycoplankton interactions with their hosts and predators may be changing across seasonal scales. Co-occurrence networks are used to assess patterns of co-occurrence between taxa and, using knowledge on their ecology and their resulting network statistics, infer potential associations or interactions (Freilich et al. 2018). We hypothesize that the ITS2 and V7-V8 primer set will capture a broader diversity of fungi than the V9 primer set. Secondly, we hypothesize that the abundance of Chytridiomycota and Rozellomycota will lag behind their host, increasing in the early winter when their green algae hosts may be more vulnerable. Prior studies have also shown green algae chla concentrations increasing in the early winter, likely as a physiological response to lower light (Patriarche et al. 2021). Thus, these algae also represent much more nutrient rich substrates for mycoplankton to target. Lastly, we expect that the co-occurrence networks will show differences in the community between seasons, including potential predators, prey, and competitors to these mycoplankton. We hypothesize that the centralities, or importance, of Rozellomycota and Chytridiomycota will increase in the winter as their zoospores would provide an alternative carbon pathway between phototrophic algae and predatory protists.

2) Methods

Study Site.

The McMurdo Dry Valleys (MDV) are positioned within the Transantarctic Mountains between the Ross Sea and the East Antarctic Ice Sheet (77–78°S, 160–164°E). Seasonality in the MDVs is extreme, with a four-month period of 24-h sunlight and four months of 24-h darkness, both of which are flanked by two months of twilight. The MDV are characterized as a polar desert, with Lake Bonney, the subject of this study, receiving less than 25 mm yr^{-1} of precipitation on average in the form of snow. However, most soils and perennially ice-covered lakes remain barren of snow due to katabatic winds (Green and Lyons 2009).

Lake Bonney is located at the foot of Taylor glacier towards the southern end of the Taylor Valley (Green and Lyons 2009; Obryk et al. 2020). Lake Bonney is 7 km long and 0.9 km wide, with a steep topography surrounding it. The Lake is split into two basins, West Lobe Bonney (WLB) and East Lobe Bonney (ELB), separated by a narrow sill which forms a channel at ~17.7 m (Obryk et al. 2020). WLB is fed largely by subglacial streams from Taylor Glacier. This stream likely contributes to high salinity and dissolved solids in the deeper waters. On the other hand, ELB gains a substantial fraction of its water from WLB (Obryk et al. 2020) through the narrow sill. These two lobes remain isolated during the winter. During the summer, ice within the sill melts enough to allow flow between these lobes. As such, this channel allows the advective mixing of the epilimnion between them, though suboxic waters below the chemocline are unaffected by this mixing and remain chemically distinct (Obryk et al. 2020; Mitchell and Takacs-Vesbach 2008). With no outlet streams and few ephemeral streams feeding into each lobe during the summer, these lakes are closed basins with limited allochthonous carbon sources (Green and Lyons 2009).

Sampling.

Sampling was performed during 2014-2017, with samples collected for four austral winters. The autonomous sampler suite was composed of four McClane laboratories instruments: A sediment trap, a phytoplankton sampler (PPS), a Remote Access Sampler (RAS), and a modified Ice Tethered Profile (ITP). These samplers are described in greater detail in (Winslow et al. 2014) though a brief summary of each instrument is provided in table 1. In short, these samplers allowed for the collection of various samples along a time series, including nutrients (C:N,

POC), Chlorophyll Fluorescence (Serving as a proxy of algal abundance), DNA, and other chemical & physical parameters. The samples collected regularly, with the sediment trap collecting every 3 weeks, the ITP daily at solar noon, the PPS every 18 days with three duplicate events, and the RAS every 9 days with five duplicate events. Samples for DNA were also collected every 2 weeks on combusted GF/F filters and preserved in sucrose lysis buffer (Mitchell and Takacs-Vesbach 2008).

Extraction and sequencing of DNA.

DNA was extracted and sequenced earlier as part of the ALPS project. Genomic DNA was extracted and purified using a custom cetyltrimethylammonium bromide (CTAB) and phenol-chloroform-IAA extraction protocol as described in Mitchell and Takacs-Vesbach (Mitchell and Takacs-Vesbach 2008). PCR was performed as described according to manufacturer protocol. The accession number for the ALPS samples is PRJNA1251086. The V9 region of the 18S rRNA gene was amplified with the primer pair 1391F (5'- AAT GAT ACG GCG ACC ACC GAG ATC TAC ACT ATC GCC GTT CGG TAC ACA CCG CCC GTC-3') and EukBr (5'-CAA GCA GAA GAC GGC ATA CGA GAT XXXXXXXXXXXX AGT CAG TCA GCA TGA TCC TTC TGC AGG TTC ACC TAC-3') (Amaral-Zettler et al. 2009; Stoeck et al. 2010).

The resulting sequence tables from this data was then assigned taxonomy using the PR2 Database V 5.0.0 (Guillou et al. 2013). Community structure was analyzed using the phyloseq (McMurdie and Holmes 2013) (V 1.46.0) R package as well as the microeco (Liu et al. 2021) R package (V 1.13.0).

Nextera sequencing of fungal ITS and V7-V8 region of 18S rRNA.

Preliminary analysis of the archived sequence libraries using the V9 18S rRNA primers indicated that fungal sequences were poorly represented. Therefore, a subset of archived DNA samples from the 2015 ALPS samples were selected for resequencing to improve the recovery of fungal sequences. Two primer sets were used, one targeting the V7-V8 Region of the 18S rRNA and one targeting the ITS2 region of the ITS region located between the SSU rRNA and the LSU rRNA (S. Chen et al. 2010). The V7-V8 region of the 18S rRNA gene was amplified with the primer pair NEX-FF390 (5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CGA TAA CGA ACG AGA CCT-3') and NEX- FR1 (5'-GTC TCG TGG GCT CGG AGA

TGT GTA TAA GAG ACA GAI CCA TTC AAT CGG TAI T-3') (Rojas-Jimenez et al. 2017). The ITS2 region was amplified with the primer pair NEX- IlluAdp_ITS31 (5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG CAT CGA TGA AGA ACG CAG-3') and NEX-IlluAdp_ITS4 (5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACA GTC CTS CGC TTA TTG ATA TGC-3') (Marchetta et al. 2023; Tedersoo et al. 2015). The Nextera XT kit was used to prepare for sequencing following manufacturer instructions. The amplicons were sequenced on an Illumina MiSeq.

18S V7-V8 sequences were processed via the DADA2 pipeline (Callahan et al. 2016). The ITS2 sequences were processed using the DADA2 pipeline for ITS as described in the DADA2 ITS Pipeline Workflow (https://benjjneb.github.io/dada2/ITS_workflow.html). In summary, sequences were not filtered due to the variability in the size of the ITS region. Instead, the primers are identified and cut out of the resulting sequences before taxonomic assignment via the UNITE database. Community structure as analyzed using the phyloseq R package (V 1.46.0) as well as the microeco R package (V 1.13.0).

Functional taxonomy assignment.

Guilds refer to the classification of species based on their shared means of resource acquisition (Simberloff and Dayan 1991). In the case of this study, we focused on means of carbon acquisition and defined these as a taxa's Trophic Mode similar to the annotation Funguild (Nguyen et al. 2016) uses. To assign trophic guilds to taxa identified by sequencing, two methods were used. For the fungal sequences, the Funguild (Nguyen et al. 2016) database was used. Due to limited cultured representatives in the Funguild dataset, Chytridiomycota, Rozellomycota, and Aphelidiomycota were manually filled based on current literature; as such, Chytridiomycota are denoted as Pathotroph-Saprotroph, Rozellomycota as Pathotrophs, and Aphelidiomycota as Pathotrophs as well (Kaczmarek and Boguś 2021; Letcher and Powell 2018; 2019). For the algal database, the PR2 Mixoplankton database was used to assign function to algal taxa where possible (Mitra et al. 2023). Otherwise, literature review of the taxa that was found to be significant in the network was used to assign taxonomy. When possible, Encyclopedia of Life (EOL) and the World Oceans Registry of Marine Species (WORMS) (Parr et al. 2014; IMIS, n.d.) were used, and primary literature was used in other cases. As such, the

confidence of trophic mode for predatory and bacterivorous taxa is of lower confidence. Definitions of trophic modes mentioned in this paper are included in table 2.

Sequence data processing. Raw sequencing data was rarefied for downstream analysis. There is great debate as to whether data should be rarefied or not as rarefaction can reduce sensitivity, add artificial uncertainty, and can be considered statistically inadmissible (McMurdie and Holmes 2014). However, recent work has begun addressing these concerns, suggesting the benefits of performing multiple iterations of rarefaction to address artificial variation (Cameron et al. 2021). To that end the MIRLYN package was used to rarefy the sequence data. In brief, the data was clustered by lake and rarefaction curves were generated. From this, a sampling depth of 2,500 was determined as ideal for both the V9 and V7-V8 primer set. MIRLYN rarefaction was performed and the resulting OTU tables combined and averaged. As the microeco package does not function with non-whole numbers, These resulting OUT tables were all multiplied by an arbitrary value (100) to ensure that rounding would not remove samples with counts falling between 0-1 in the original dataset. This shouldn't impact downstream analysis or comparison given that it is an internal comparison.

Network construction.

Co-occurrence networks were constructed using the microeco R package. Rarefied OTU tables from the algal database were combined with fungal sequences from the rarefied OTU tables from the fungal database. This was done with the goal of exploring how fungi specifically associate to algae and other protists identified in that dataset. These OTU tables were used to calculate correlation using the sparCC correlation method at the OTU level. This method accounts for the unreliability of using compositional data to generate networks (Friedman and Alm 2012). Networks were constructed via a correlation-based approach (p-val= .05, correlation threshold of .6). Networks were visualized in R using the ggraph package (Penderson 2024) (V 2.2.1).

Diversity analysis, network feature comparison, and network robustness comparison.

Alpha diversity metrics were calculated using the microeco package. Shannon and Chao1 were calculated for each lake via anova with an alpha value of .05. Bray-Curtis Dissimilarity was calculated by grouping samples based on season & lake. Differential Abundance was calculated

using the lefse algorithm to an alpha score of .05 with 250 bootstrap replicates. Due to limited representatives from autumn and spring, only summer and winter were used in this analysis.

Intramodule and intermodule (Z score & P score respectively) connectivity were extracted from the resulting networks. These scores were used to assign taxa roles to each node as described in Guimerà et al (Guimerà and Nunes Amaral 2005). In short, nodes can fall within peripheral, connector, and module roles. Network Metrics (Modularity, Betweenness centrality, etc.) were also calculated using microeco and normalized Degree, Betweenness Centrality, and Closeness Centrality were calculated for inter-network comparison. Average Betweenness Centrality, Closeness Centrality, and Eigenvector Centrality were calculated across each season for ELB and WLB for every trophic guild present in the networks. These centrality measures relate to how well connected nodes are in their networks. Detailed descriptions are present in table 3. In brief, Betweenness Centrality refers to the ability of nodes to act as bridges within networks, with high betweenness centrality nodes facilitating information flow. Closeness Centrality measures how close a node is to all others, with closer nodes spreading changes quicker along a network. Eigenvector Centrality measures how connected a node is to important nodes, thus those with high Eigenvector Centrality are important towards networks stability and connectivity. When possible, Kruskal Wallis tests ($p>.05$) were performed to assess the significance of these seasonal differences.

Network Robustness and Vulnerability describe how vulnerable networks are to the loss of Global Network Efficiency (Bellingeri et al. 2020; Yuan et al. 2021). In the context of ecological networks, the loss of Global Network Efficiency equates to a loss of community structure. These metrics were calculated and explored using the meconetcomp package (Liu et al. 2023) (V 0.6.1). An ANCOVA was performed to compare differences between the resulting Robustness Regressions.

3) Results

Sequence Data Composition pre and post rarefaction.

To achieve maximum recovery of fungal sequences, this study employed two new primer sets for sequencing as well as the original ALPS dataset. The archived ALPS dataset used the EMP primer set which targets the V9 region of the 18S Ribosomal Subunit. For the resequencing performed as part of this study, Primers targeting the ITS2 region and the V7V8 region of the 18S Ribosomal Subunit were used to better target aquatic fungi. Both of these primers were used in prior studies of Antarctic lakes ((Rojas-Jimenez et al. 2017; Marchetta et al. 2023).

The Archived ALPS (V9) data identified 1,752,741 reads with 1399 OTU's, the V7V8 identified 249,801 reads with 465 OTU's, and the ITS primer set identified 84,764 reads with 533 OTU's (Supplementary Figure 1). Each primer set showed signficant differences in the distribution of eukaryote taxa, emphasizing the effect of primer bias. At the subdivision level, the ALPS dataset was dominated by reads from Gyrista (63.43%) while showing limited fungal reads (2.47%). Gyrista is a phylum of heterokont protists, dominated by Ochrophyta, which has been detected in high abundance in Lake Bonney in previous studies (eg: Li and Morgan-Kiss 2019). Effectively, a large portion of these reads belong to Ochromonadales with some psuedofungi (Peronosporomycetes) present in very low abundances. The V7V8 dataset identified additional fungal reads (33.63%) as well as high abundance of Cercozoa (30.23%) and Gyrista (17%) (supplementary figure 1C, 1D). Lastly, though the ITS dataset detected only Fungi, about 74.59% of these reads were unidentified at the Phylum level (equivalent to Class in the PR2 dataset) (Supplementary Figure 1E, 1F). A previous study on the effectiveness of these primers show similar results (Tedersoo et al. 2015), however, this study showed great coverage on various fungal classes which this dataset does not have. A study in arctic lake Knudsenheia, Svalbard which also used the same primer set found similarly high abundance of uncharacterized reads (Marchetta et al. 2023) but even this was anomalous compared to the other lakes studied. Given the high abundance of uncharacterized reads, the ITS dataset was not included in downstream network analysis. Given the high abundance of algal reads in the V9 primer set and the high abundance of fungal reads in the V7V8 dataset, these were referred to as the Algal and Fungal community, respectively, in the

continuing sections. Both the Algal and Fungal community sequence databases were utilized for further analyses.

After rarefaction, 3 samples from the fungal dataset were dropped due to low sequence counts, representing one from WLB and two from ELB. These removals resulted in ELB missing data from January and December, with WLB missing data from February for the fungal community. Of the Algal community dataset, 4 samples were dropped, 2 from WLB and 2 from ELB. However, these samples are not within the sampling year this study focused on.

Seasonal differences within Algal and Fungal communities in ELB and WLB .

Community richness and diversity were explored using the Chao1 Index and Shannon Diversity Index, respectively, using the rarefied data to calculate both metrics. The algal community showed significant differences (Kruskal Wallis Dunn Test, $p < 0.05$) in species richness between summer and winter in both lakes (Figure 1A), with summer showing greater species richness in both lakes. Shoulder seasons, referring to autumn and spring, clustered closer to winter in richness score, though did not significantly differ from either summer or winter (figure 1A). No significant differences in species diversity were found between seasons (figure 1B). However, it is of interest to note that autumn and spring diversity scores closer to summer and winter, respectively (Figure 1B). No significant differences in species richness and diversity were detected for the fungal community or for the ITS primers (figure 1C-1F), with summer and winter appearing similar across both lakes. Further, it was interesting that both lakes showed similar trends between ELB and WLB.

Algal and fungal communities exhibited distinct seasonal trends in community composition. (figure 2). In both lakes, Chrysophyceae makes up a large percentage of the algal community reads (~70-75% in the winter) (figure 2A, B), with Prymnesiophyceae, Chlorophyceae, and Eustigmatophyceae showing higher abundances in the summer (figure 2A, 2B). To assess the differences in taxonomic composition across seasons, bray-curtis dissimilarity was calculated between summer and winter for each lake on rarefied data. For both the algal and fungal communities, spring and autumn are highly dissimilar (figure 3A-D). For the algal community, winter samples cluster closer together which suggests they are less dissimilar from eachother. Summer samples show higher dissimilarity in both lakes, with

December samples clustering closer to winter samples (figure 3A and 3B respectively). ANCOVA analysis confirms that ELB shows significant seasonal differences in the taxonomic composition of their algal community, with summer and autumn differing significantly from winter. WLB is just barely not significant (p-adjusted 0.054) (supplementary table 1).

The high abundance of Chrysophyceae is also reflected in the fungal community (figure 2C, 2D) though only during the autumn and winter months. The fungal community shows higher abundance of Rozellomycota and Chytridiomycota in the winter, with Rozellomycota dominating ELB and Chytridiomycota dominating WLB (figure 2C, 2D). WLB also shows a higher abundance of another algal parasite, Aphelidiomycota, from January to may (late summer to mid-winter). Interestingly, where Chytridiomycota shows higher abundance in the winter, Rozellomycota shows peaks in abundance in the late summer and middle of the winter, which suggests that the abundance of this group may be driven by different factors than Chytridiomycota. The ITS primer set reflects the higher abundance of Rozellomycota and Chytridiomycota in the winter (figure 2E, 2F), though not the peaks of Rozellomycota abundance seen in ELB during the late summer. In regard to taxonomic composition, the fungal samples do not cluster close to eachother and show high dissimilarity during the summer and winter (Figure 3C, 3D). Further, summer and winter samples appear dissimilar from each other in WLB (figure 3C) but not ELB (figure 3D). ANCOVA analysis shows that only WLB shows significant differences in taxonomic composition across seasons (supplementary table 1) with summer and winter differing significantly. Due to lack of replicates, significance could not be assessed for autumn and spring. Similar trends are seen in the ITS primer set, though no significant differences between seasons were detected for this primer set (Figure 3 E,F).

Winter communities exhibit higher Pathotrophic fungi in WLB.

Given the differences in species diversity, richness, and taxonomic composition found in both the fungal community (V7V8 Primer set) and algal community (V9 Primer set), differential abundance scores were calculated using Lefse LDA scores. This was done with the goal of assessing whether any fungal or algal taxa showed significant differences in their seasonal abundance. Due to limited replicates for spring and autumn, these seasons were

omitted from this analysis. For the algal community both lakes, Cryptophyceae, Bolidiophyceae, Bacillariophyceae, and Chlorophyceae show significantly higher abundances in the summer when compared to winter (figure 4A, 4B). Chrysophyceae shows higher abundance in the winter when compared to the summer, hence its negative LDA score. For the fungal community, only fungal taxa in WLB exhibited significant seasonal differences in abundance. Rozellomycota and Chytridiomycota are both significantly more abundant in the winter when compared to the summer. Additionally, Chrysophyceae is also found to have significantly higher abundance in the winter when compared to summer (figure 4C, 4D) in WLB. Rozellomycota and Chytridiomycota both represent pathotrophic taxa, indicating that winter conditions favor the presence of these taxa. Both datasets also show a taxonomic group labelled "c__". These are taxa that were not characterized at a class level. A majority of these reads belong to the Subdivision Cercazoa, which represent a diverse group of amoeboids and flagellates (Khanipour Roshan et al. 2021). Given the metabolic diversity of this group (Predators, Autotrophs, Parasites) and the limited taxonomic information, interpretation of their differential abundance is difficult.

Network members reflect higher abundances of pathotrophs and mixotrophs

To understand community patterns of co-occurrence and the overall stability of these associations between seasons, ecological co-occurrence networks were generated (Figure 5). These were done using the SparCC algorithm which accounts for the compositional effects of both datasets (see methods). These networks were generated using the combined algal community data and fungal community data to capture potential associations within and between the datasets. These were done at an OTU level to identify important sequences. These OTU's also had taxonomic information as well as trophic mode information appended to them. Downstream analysis of their statistics (centralities, Z & P scores, etc.) considered their taxonomic information as well as their expected trophic mode. Networks were generated for WLB summer (WS), WLB winter (WW), ELB summer (ES), and ELB winter (EW).

The summer networks on average showed more nodes (47 for WS and 54 for ES) than in the winter (37 in WW and 41 in EW). Looking at the trophic modes identified in the nodes, we see that summer networks generally show a more even spread between the trophic modes

defined in table 2 (Figure 6). Of note, ELB shows slightly greater abundances of mixotrophs in the summer when compared to WLB. Winter communities have higher abundances of pathotrophs in both ELB and WLB, with the Pathotroph group (made up of largely Rozellomycota) showing the highest abundance (Figure 6). Interestingly, the abundance of predators in the networks decreases in the winter compared to summer. Similarly, mixotrophic members also decrease in the winter which is unexpected given that this trophic mode is expected to be more advantageous. It is possible, given losses in species richness observed in figure 2, that the networks are reflecting the dominance of a few species which outcompete other mixotrophs.

Network topological features are lake-specific but show similar seasonal trends.

The generated networks show differences in the topology of their detected communities (Figure 5, Table 4). The topological features we explored were Average Degree, Average Path Length, Centralization, Clustering Coefficient, Density, Heterogeneity, Modularity, and +/- edges. These are described in greater detail in Table 3. The features which best characterize the differences in connectivity in these networks were Centralization, which refers to how central its most central nodes are, Clustering Coefficient, which refers to how closely nodes tend to cluster, and Modularity, which refers to how strongly the network is divided.

WLB Networks (Table 4; WS, WW) show lower centralization than ELB networks (Table 4; ES, EW) (table 4) which can also be observed by the "star" network structure both ES and EW show. WLB networks also show higher clustering coefficient than ELB. Together, this suggests that WLB communities form distinct modules which are not as strongly connected. This is observed within the networks (figure 5), with WS showing the clearest example of this (Figure 5A). Along the top of the network, a cluster consisting of mostly phototrophs and mixotrophs show many connections amongst themselves. The same is observed towards the right of the network, with this cluster consisting largely of predators and bacterivores. Thus, it appears that the network is grouping based on the trophic mode of the identified node, with pathotrophic taxa (represented in purple) being present in all clusters. Again, ELB networks instead display a "star" network structure which indicates networks which are more strongly connected to few central nodes, in this case bacterivorous

taxa. Interestingly, these structures are more defined in the summer than they are in the winter (Figure 5). This may be due to changes in the modularity, intensity of division, of the networks.

Networks in both lakes decrease in modularity from summer to winter (~0.3 to ~0.1) (table 4), suggesting that summer communities are defined by modules with stronger intra-module connections (ie. higher connectivity between nodes within a module) and with overall lower inter-module connectivity, or that they are less connected with the rest of the network. This is also supported by nodes with higher among-module connectivity observed in the winter (figure 7). Among-module connectivity refers to the inter-module connectivity. In an ecological context, networks showing higher among-module connectivity show communities that have alternative pathways to share resources which generally means these networks are more stable. Further, the ratio of co-occurrences (+ edges) and co-exclusions (- edges) is more balanced in the winter (Table 4, Figure 5). This suggests that the winter networks are showing more complex patterns of association than in the summer.

ELB & WLB show differing trends in network stability across seasons.

The generated co-occurrence networks suggest that winter networks are showing less divided communities (lower modularity) with more complex patterns of association (+ edge to – edge). In tandem with the higher abundances of pathotrophic taxa, this suggests that these communities may be more strongly influenced by the activity of the mycoloop. To further explore this hypothesis, we sought to assess the stability of the network. Generally, networks that are closely connected (lower modularity, higher centralization) will be more stable as the network structure is more complex and the loss of one node will not jeopardize the networks overall connectivity, or global efficiency. In an ecological context, communities with more complex trophic interactions should result in more stable networks. Given that the mycoloop adds an alternative carbon pathway, and thus another layer of trophic interaction, we hypothesized that the winter networks would show greater stability.

To further explore differences in network stability, two metrics were used: robustness and vulnerability. Robustness is the ability of a network to resist losses to its connectivity (global efficiency) either through failures (loss of node diversity) or attacks (loss of central nodes). This is measured in the loss of global efficiency upon removal of a certain fraction of nodes,

with the slope of the resulting regression defining robustness; the larger the slope, the less robust a network is. Similarly, vulnerability refers to the loss of global efficiency upon the removal of a node. This is calculated differently from robustness (check methods) but can serve to complement the analysis. Given the network features (table 4), we expected that summer networks would show lower robustness and higher vulnerability.

Upon inspection of initial global efficiency, before node removal, ELB networks (ES, EW) initially exhibited higher global efficiencies relative to WLB networks (WS, WW; Figure 8). It is possible this has something to do with the higher centralization seen in ES and EW (Table 4); however, both ELB networks exhibited faster losses in global efficiency as removal ratio increased (Figure 8A, 8C). Further, ELB summer and WLB summer show different initial global efficiency and resulting regression (Figure 8D, 8E), with WLB summer showing far greater robustness than ELB summer. An ANCOVA analysis comparing the removal ratio of nodes to global efficiency found that networks, that is to say the combined effects of seasonality and lake, had a significant impact on the slopes of the resulting regression (table 5). The resulting regression of the attack model (Figure 8C) shows that ELB summer was the least robust network and WLB summer the most robust network. This result was unexpected given that both lakes showed higher modularity and positive edges (table 2) during the summer when compared to the winter. Modularity and higher fractions of positive edges have been implicated in lower network stability. Interestingly, the robustness of Winter networks appears to have more comparable slopes and fall in between ELB summer and WLB summer (ES and WS, respectively; Figure 9C). Upon performing a Tukey Test to compare slopes, we found that all slopes were significantly different except for the slopes of WW & EW and WS & WW (Table 6). It seems that ELB shows greater differences in robustness between summer and winter, while WLB shows more robust networks across both seasons. Similarly, the mean vulnerability scores of the networks also show significant differences in vulnerability across all networks, with ELB showing higher vulnerability than WLB (figure 10). This reinforces that ELB networks are less stable and are expected to lose connections and network efficiency under disturbance, particularly when losing their most connected taxa.

Pathotrophic Taxa show significantly higher centralities in the winter.

The lake specific trends in network stability observed above may be indicative of differences between the connectivity within the networks. It is possible that node specific connectivity features such as Eigenvector Centrality, Closeness Centrality, etc. are contributing to stability. To evaluate how specific taxa may influence connectivity within the networks between seasons, changes in centrality scores were compared across several major taxa (Figure 11?). The network metrics that were assessed were Eigenvector Centrality (EC) (ie. How important the node and its neighbors are), Normalized Closeness Centrality (NCC) (ie. how close nodes are to others), Normalized Betweenness Centrality (NBC) (ie. how often nodes fall on the shortest path), and Normalized Degree (ND) (ie. the number of connections to the node).

In both lakes, all identified taxa showed increases in NCC during the winter, which is expected given the lower modularity observed in the winter, with Rozellomycota, Choanoflagatea, and Prymnesiophcyeae showing significant differences (Figure 10C, D; wilcox test, $p<0.05$). This suggests that these taxa are important for maintaining network structure, with Prymnesiophcyeae, a mixotroph, showing amongst the highest scores. Prymnesiophyceae maintains high NBC and ND in the winter for both lakes which emphasizes the importance of mixotrophs to maintaining community structure (Figure 10E-H). Rozellomycota shows significant seasonal differences in NBC in both lakes, being higher in the summer in WLB and higher in the winter in ELB (10E, F). Though more central in the winter, it would appear the extent to which Rozellomycota acts as a bridge in the networks is lake dependent (Figure 10E, F). In WLB, Rozellomycota bridges more nodes in the summer based on its NDC and NBC. Contrarily, Rozellomycota shows higher EC and NDC in ELB during the winter which implies they are connecting more taxa during the winter months.

Table 1 Core instrumentation used as part of the ALPS project. This includes core instrumentation which are part of the greater LTER project, Sediment Traps, the Ice-tethered profiler (ITP), the RAS, and the PPS. For this study, eDNA samples collected by the PPS were used. The PPS collected samples every 18 days with 3 duplicate events.

Autonomous Lake Profile Sampler (ALPS) sensors and samplers		
Instrument	**Details**	**Frequency of Measurement**
Core instrumentation	Lake Level, Ice Thickness, Underwater PAR	15 minutes
Sediment Traps	Sediment Fluxes; N, C, P Fluxes	3 weeks
ITP	Ice Tethered Profiler; Salinity, Temperature, CO2, O2	Daily at solar noon
RAS	Water Chemistry, chla concentration	9 days (5 duplicate events)
PPS	Collected & preserved eDNA samples	18 days (3 duplicate events)

Table 2 Ecological Definition of Trophic Modes as defined by this paper, the PR2 Mixoplankton Database, and Funguild. The identified Trophic Modes focus on how the taxa interact with their prey or chosen carbon source.

Ecological Defintion of Trophic Modes		
Trophic Mode	**Definition**	**Source**
Bacteriovore	Heterotroph feeding primarily on bacteria and small protists.	
Predator	Heterotroph feeding primarily on other protists and bacteria.	
Phototroph	Protist capable of acquiring carbon via photosynthesis.	
Mixotroph	Phototroph capable of acquiring organic carbon either by Osmotrophy or Phagotrophy.	Mitra et al., 2023
CM	Constitutive Mixotrophs. Mixotrophs which harbor their own photosynthetic apparatus.	Mitra et al., 2023
pSNCM	Plastidic Specialist Non-Constitutive Mixoplankton. Mixotrophs which steal photosynthetic apparatus from specific phototrophic prey.	Mitra et al., 2023
GNCM	General Non-Constitutive Mixoplankton. Mixotrophs which steal photosynthetic from many types of phototrophic prey.	Mitra et al., 2023
eSNCM	Endosymbiotic specialist Non-Constitutive Mixoplankton. Mixotrophs harboring endosymbionts with photosynthetic apparatus.	Mitra et al., 2023
Saprotrophs	Heterotroph which feeds by degrading dead host cells and other detritus.	Nguyen et al., 2016
Symbiotrophs	Heterotroph which feeds by exchanging nutrients with host cells.	Nguyen et al., 2016
Pathotrophs	Heterotroph which feeds by harming host cells. Includes Pathogens and Parasites.	Nguyen et al., 2016
Multiple Assignments	Some taxa in the Funguild database have representatives spanning multiple trophic modes. As such, some taxa are identified by all possible modes.	Nguyen et al., 2016

Table 3 Definitions and details of network features and statistics including Betweenness Centrality, Closeness Centrality, Eigenvector Centrality, Robustness, and Vulnerability.

Network Features and Statistics		
Feature	**Description**	**Source**
Closeness Centrality	Proximity of a node to all others. Calculated by the average shortest path length.	Hernandez et al. 2021; Guimerà and Nunes Amaral 2005
Betweenness Centrality	Measures how often a node falls on the shortest path between nodes.	Vicks-Major et al. 2013
Eigenvector Centrality	Measures the impact a node has on the network based on the centrality of its neighbours	Lee et al. 2022
Robustness	Refers to how robust a network is to random or targetted removal of nodes or edges. This is measured as the loss in Global Efficiency (y) upon node removal (x). The resulting regression defines the robustness, with greater slopes denoting less robust networks.	Bellingeri et al. 2020
Vulnerability	Vulnerability measures the loss of global efficiency upon node removals. Networks with high vulnerability scores are more vulnerable to losses in nodes	Yuan et al. 2021

Table 4 Network Statistics for the Generated Co-occurrence networks. Modularity is higher during the summer months for both, with other statistics differing by lake. In WLB, nodes cluster more, are less heterogeneous, and less centralized in the summer. In ELB, nodes cluster more strongly, are less heterogeneous, and less centralized in the winter.

	Average Degree	**Average Path Length**	**Centralization**	**Clustering Coefficient**	**Density**	**Heterogeneity**	**Modularity**	**+**	**-**
WS	14.55	1.68	0.68	0.54	0.32	0.64	0.3	66.96	33.04
WW	13.14	1.61	0.64	0.47	0.36	0.72	0.1	51.03	48.97
ES	6.15	1.88	0.88	0.22	0.12	1.53	0.33	68.07	31.93
EW	11.61	1.71	0.71	0.37	0.29	0.98	0.08	56.3	43.7

Table 5 Resulting Ancova assessing if the removal of nodes (remove_ratio) impacts the global efficiency (tmp1) and if the combined effects of remove_ ratio and Network cause significant changes in the global efficiency. Resulting Ancova shows significant effects on the global efficiency (tmp1) upon node removal, between networks, and node removal when accounting for the network.

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**	**Significance**
tmp1$remove_ratio	1	0.08136776	0.08136776	114.860758	7.42E-17	***
Network	3	0.03291182	0.01097061	15.4863819	5.92E-08	***
tmp1$remove_ratio:Network	3	0.04284743	0.01428248	20.1615003	1.03E-09	***
Residuals	76	0.05383866	0.0007084	NA	NA	

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Table 6 Tukey Test of the Ancova generated for the Robustness regressions. The slopes for WW and WS do not differ significantly (p=.505). Further, the slopes for EW and WW do not differ significantly (p=.059)

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**	**Significance**
WW - WS	0.02217966	0.01585774	1.39866464	0.50409974	
ES - WS	0.14925004	0.01585774	9.4118107	1.40E-14	***
EW - WS	0.06273597	0.01585774	3.95617379	0.00092619	***
ES - WW	0.12707038	0.01585774	8.01314605	1.65E-11	***
EW - WW	0.04055631	0.01585774	2.55750915	0.05898264	.
EW - ES	-0.0865141	0.01585774	-5.4556369	2.12E-06	***

Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

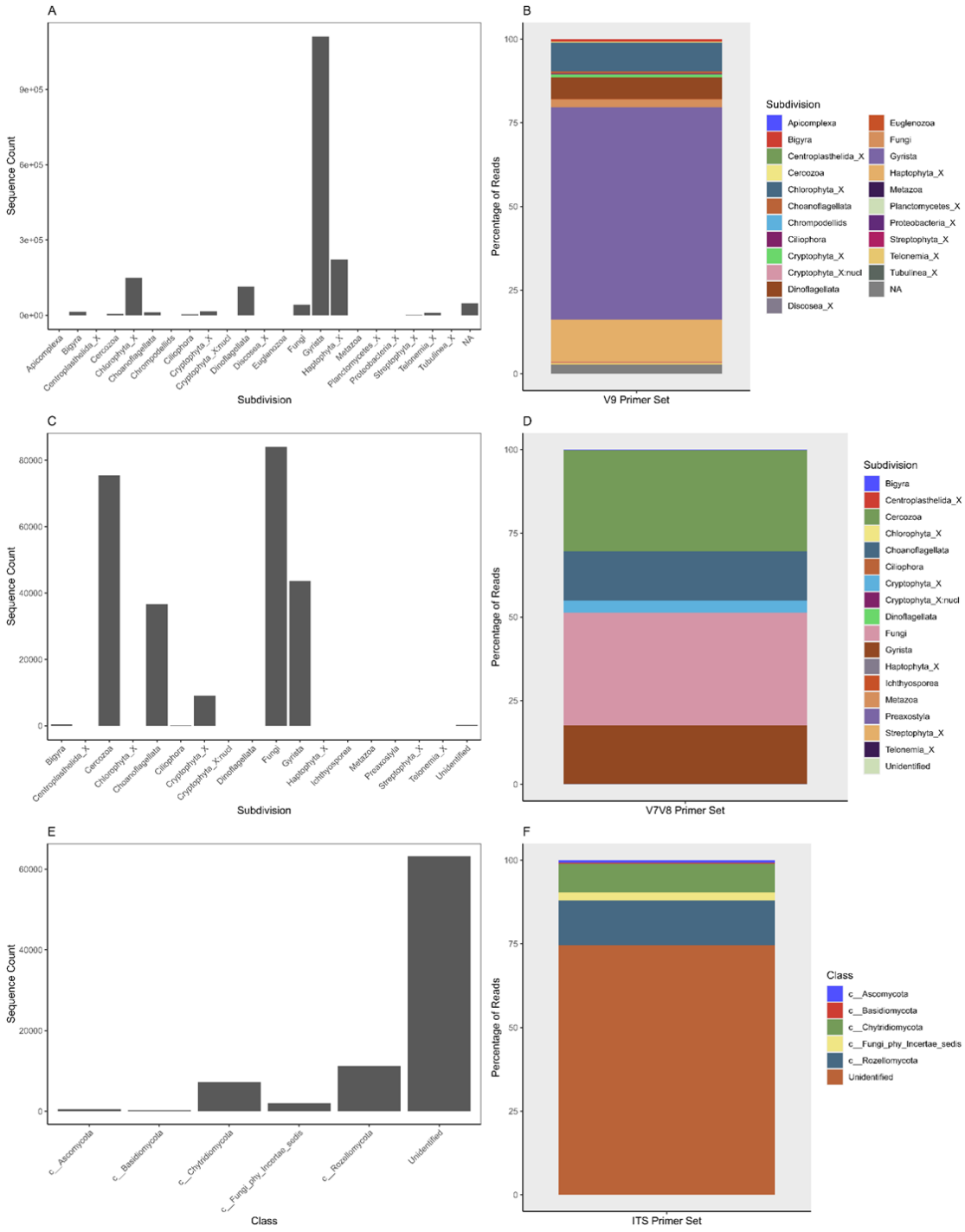

Figure 1. Data composition of resulting amplicon sequencing. The raw sequence count (A,C,E) are shown for the V9, V7-V8, and ITS primer set (A, C, E respectively) separated by subdivision for the V9 and V7-V8 and by class for the ITS primer set. Alongside them (B, D, F) is the relative percentage of reads corresponding to each subdivision, or class, for the V9, V7-V8, and ITS primer sets (B, D, and F respectively). The V9 primer set has the highest sequence count, about 1,752,741 reads with 1399 OTU's, and primarily captured Hyrista, Haptophyta, Chlorophyta, and Dinoflagellatea. The V7-V8 primer set detected 249,801 reads with 465 OTU's and primarily captured Fungi, Gyrista, and Cercozoa. The ITS primer set detected 84,764 reads with 533 OTU's and detected only fungi. Of these, a majority were unidentified at a class level. Due to high algal read count, the V9 primerset is referred to as the Algal Community and the V7-V8 primer set is referred to as the Fungal Community In subsequent results and discussion.

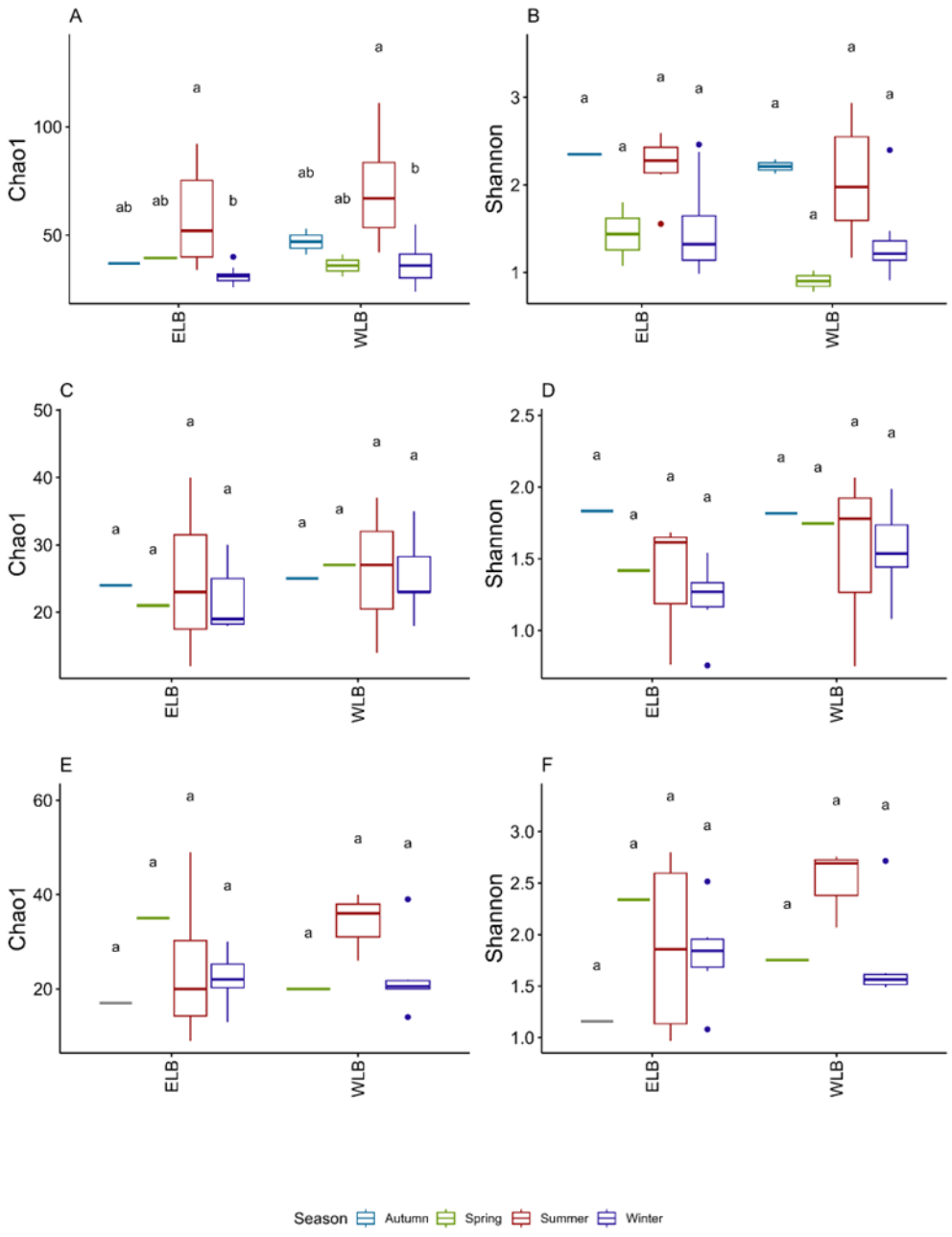

Figure 2. Alpha Diversity metrics across the three primer sets. Chao1 index (A, C, E) was used to assess shifts in species richness across seasons for each lake, and Shannon Diversity Index (B, D, F) was used to assess shifts in species diversity across seasons for each lake. The datasets are presented in the order of the Algal Community (A, B), the Fungal Community (C, D), and the ITS primer set (E, F). Kruskal-Wallis test was performed to see if scores differed by season for each lake ($p < 0.05$) and Dunn's Test ($p<0.05$) was performed to assess which group means significantly differed from each other. Significant differences in species richness were found between summer and winter in both lakes for the Algal Community. No significant differences in species richness or diversity were detected for the Fungal community or the ITS primer set.

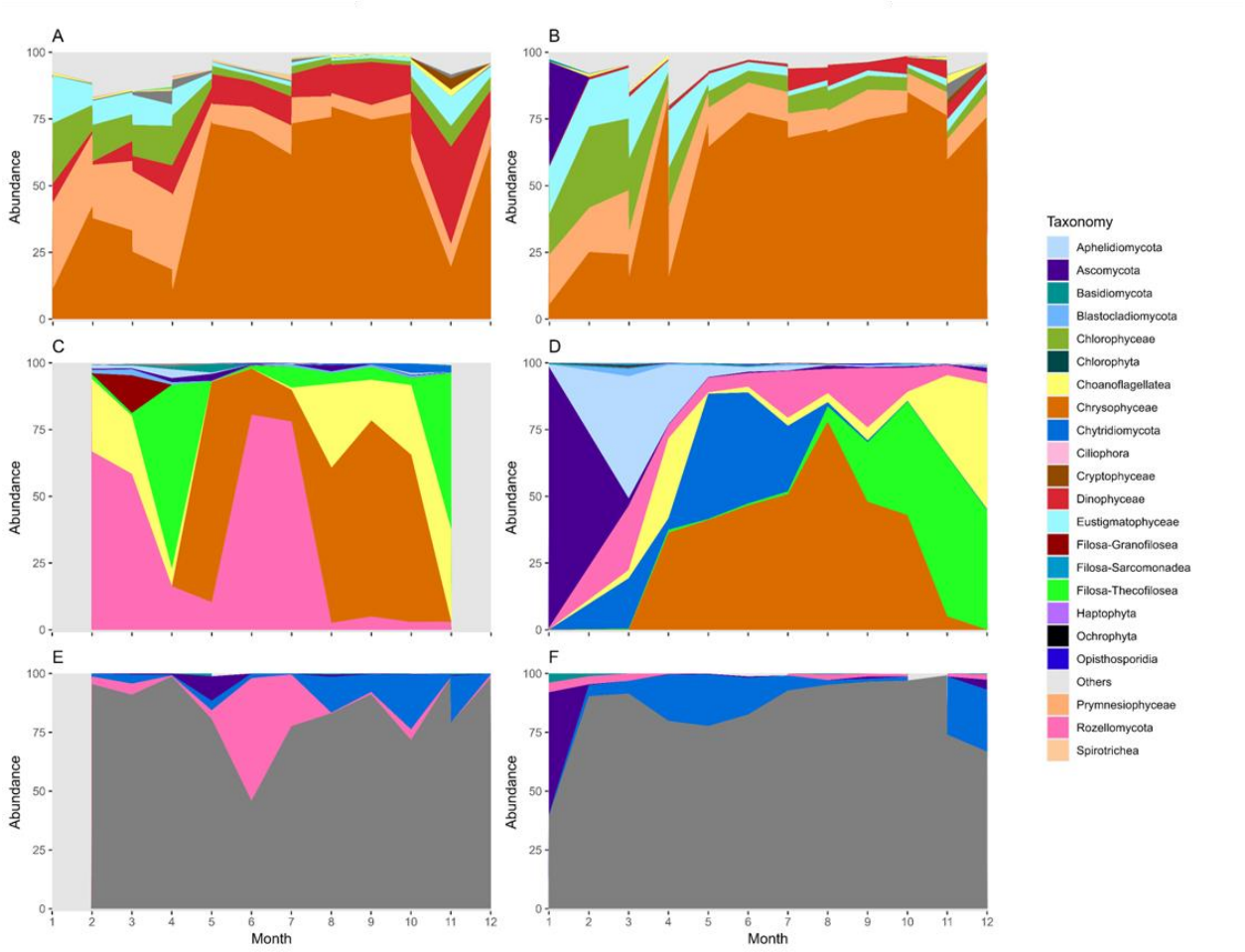

Figure 3 Relative abundance trends for the three primer sets from January 2015 to December 2015 for ELB (A, C, E) and WLB (B, D, F). Taxonomy is represented at the Class level. Dashed lines represent the shoulder seasons, with autumn spanning from March to April and spring from October to November. The datasets are ordered, top to bottom: V9, the Algal Community, (A, B), the V7-V8, the Fungal Community, (C, D), and the ITS primer set (E, F). The Algal Community shows increased abundance of Chrysophyceae during the winter across both lakes with summer showing more diverse communities. The Fungal Community shows communities that appear specific to each lake, with ELB being dominated by Rozellomycota and WLB by Chytridiomycota. The abundance of these pathotrophic taxa peaks during the winter months, though Rozellomycota also has a peak during the late summer In ELB. The ITS primer set also mirrors these abundance trends.

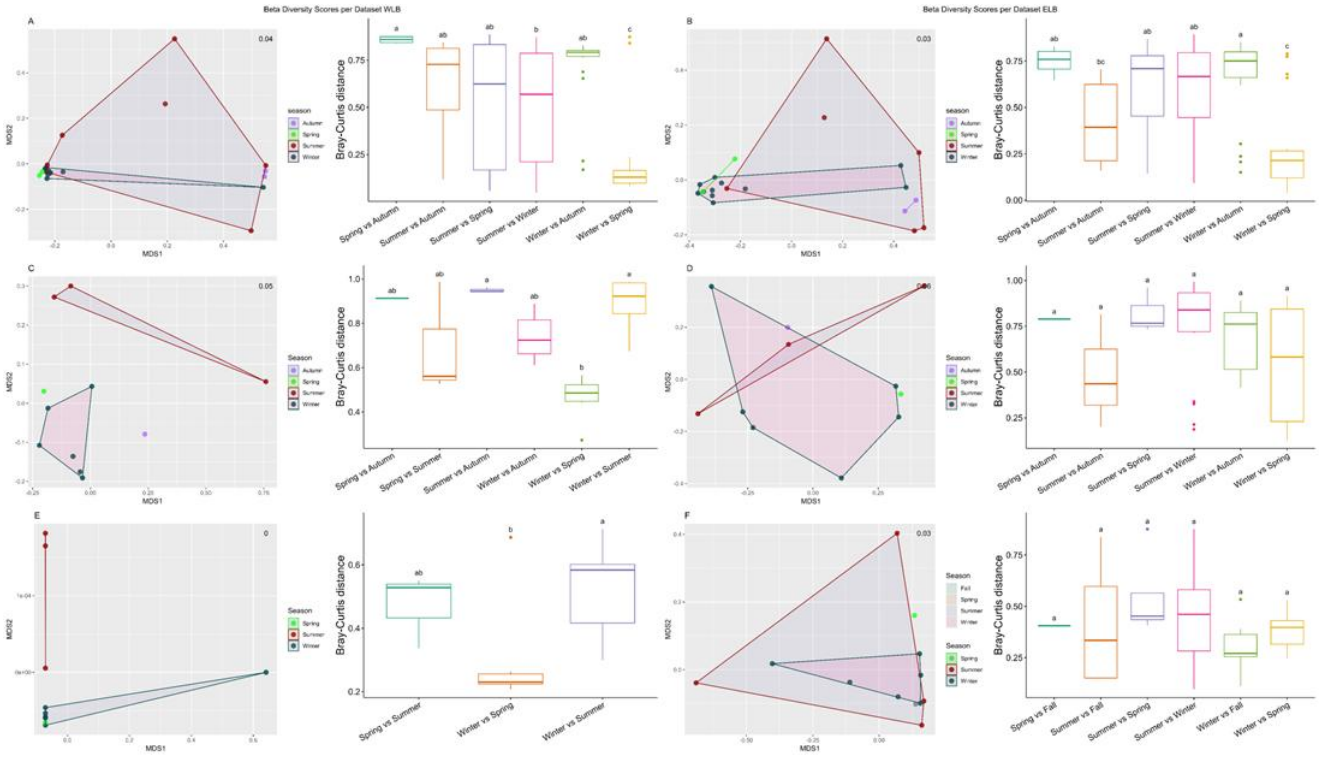

Figure 4 NMDS of bray curtis dissimilarity scores with adjoining Kruskal-Wallis-Dunn test ($p<0.05$) for WLB (A,C,E) and ELB (B,D,F). The datasets are ordered, top to bottom, V9, the Algal Community, (A, B), the V7-V8, the Fungal Community, (C, D), and the ITS primer set (E, F). The shoulder seasons, autumn and spring, show high dissimilarity in taxonomic composition from one another across all datasets. Summer and winter also show high dissimilarity, with some summer samples clustering close to each other in the Algal Community across both lakes and in ELB in the Fungal Community. Winter and Summer are highly dissimilar for the Fungal Community and ITS primer set. ANCOVA analysis confirms that both ELB and WLB show significant seasonal differences in the taxonomic composition of their algal community, with only WLB showing significant differences in taxonomic composition across seasons for the Fungal Community. Similar trends are seen in the ITS primer set, though no significant differences between seasons were detected for this primer set.

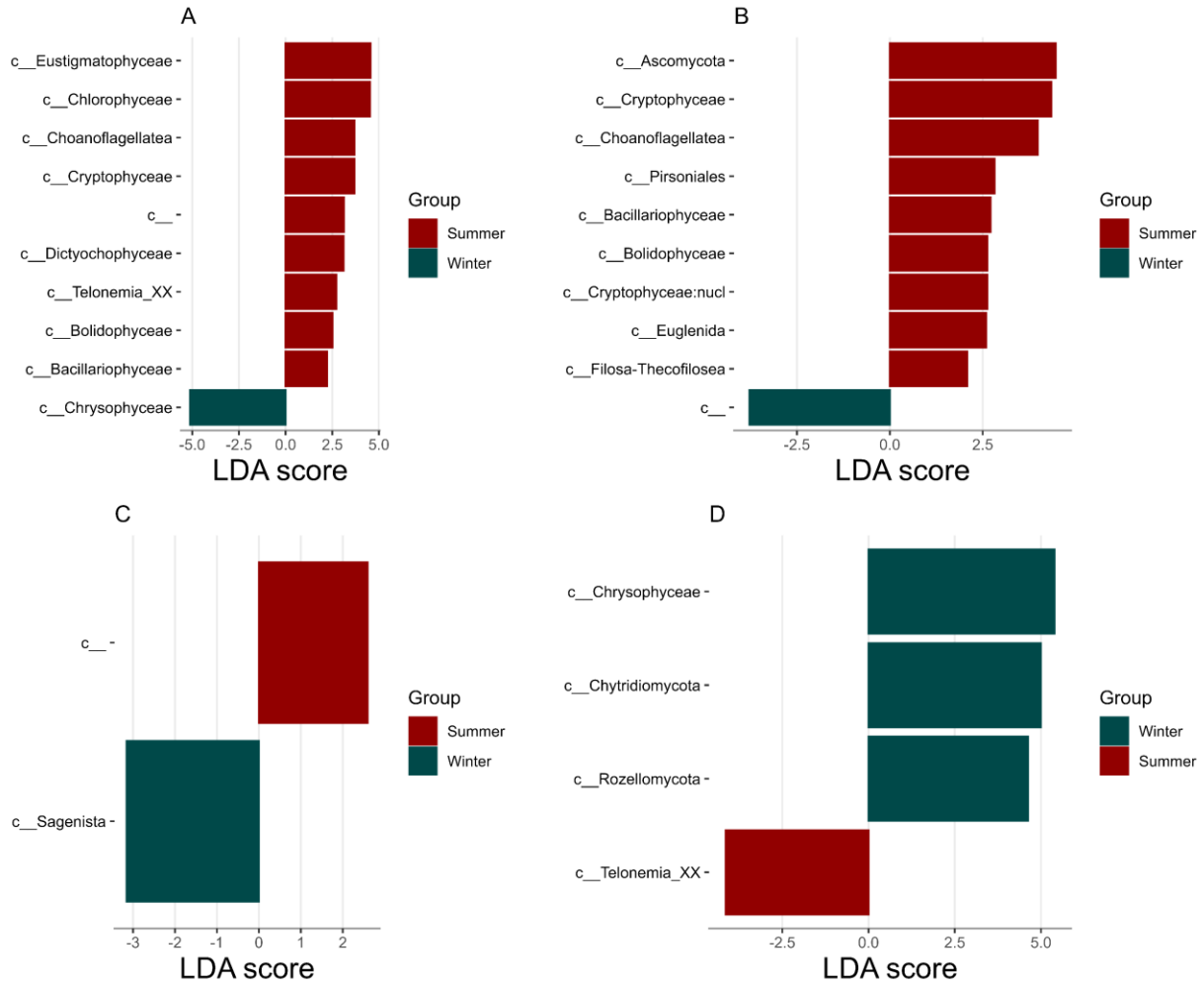

Figure 5 Lefse LDA Differential Abundance Scores for ELB (A, C) and WLB (B, D) between summer and winter. The Algal Community is presented in the top row (A, B) and the Fungal Community in the Bottom Row (C, D). The Algal Community shows Cryptophyceae, Bolidiophyceae, and Bacillariophyceae have significantly higher abundance in the summer when compared to winter across both lakes. For the fungal community, no fungi are found to significantly differ in their abundance between summer and winter in ELB. In WLB, Chytridiomycota and Rozellomycota have significantly higher abundances in the winter when compared to the summer.

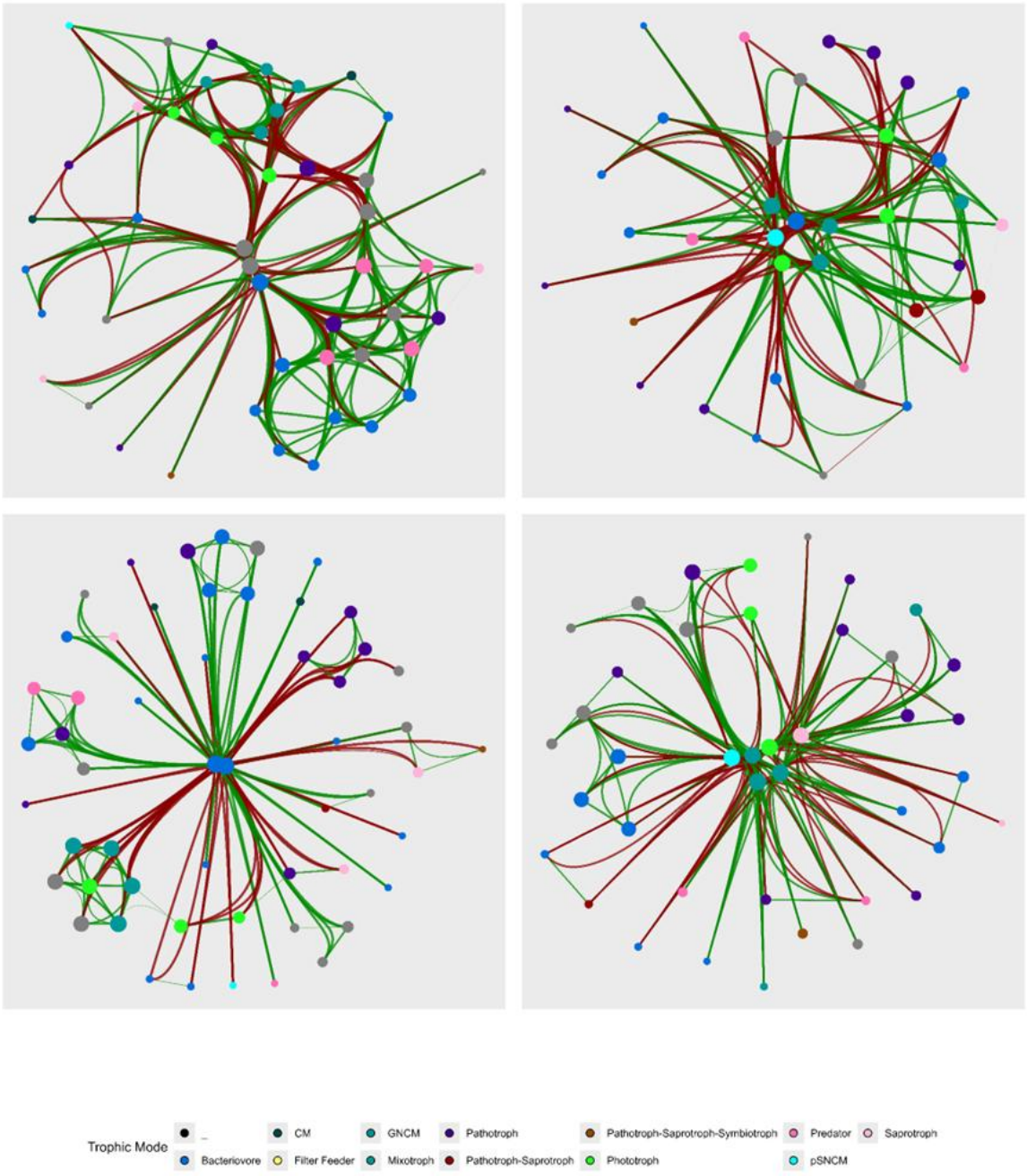

Figure 6 Co-occurrence network calculated using SPARCC and generated via spearman correlation (correlation cutoff 0.6, filter threshold 0.001, $p<0.05$). Nodes are colored by Trophic Mode. The top row represents WLB summer and winter respectively, and the bottom ELB summer and winter respectively. Edges were drawn based on their shortest paths, preserving network topography while clustering similar connections. The size of the nodes is based on their eigenvector centrality, with larger nodes showing higher eigenvector centrality. WLB networks are more modular than ELB networks. A higher abundance of bacterivores is present in the summer, with a bacterivore OTU and two unidentified OTU's forming a central cluster. In the Winter, this central cluster is formed by mixotrophic taxa. In ELB, the networks form more of a star pattern which is indicative of a more densely connected network. The central cluster is made up of bacterivores in the summer and mixotrophic taxa in the winter.

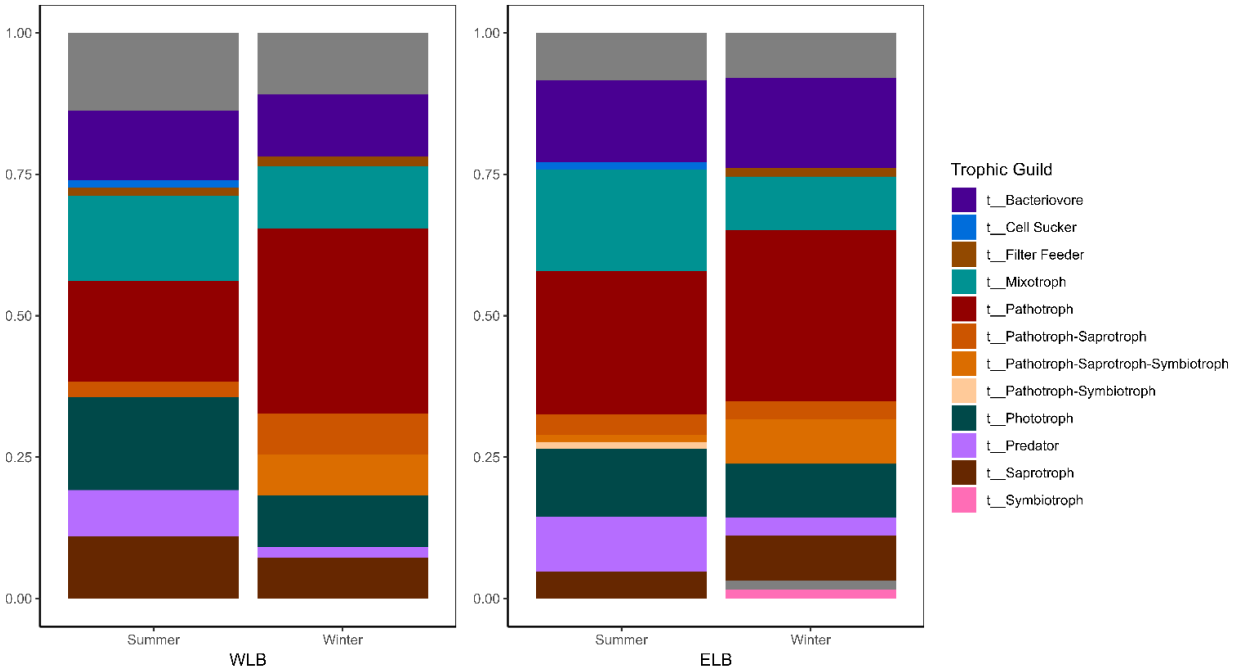

Figure 7 Relative Abundance of Trophic Guilds within the Co-occurrence networks. Gray boxes denote taxa with unknown or uncertain Trophic Modes. In both lakes, Pathotrophs show higher abundance in the winter along with Pathotroph-saprotroph-symbiotrophs. Phototrophs also see lower abundances, with predators also decreasing in relative abundance.

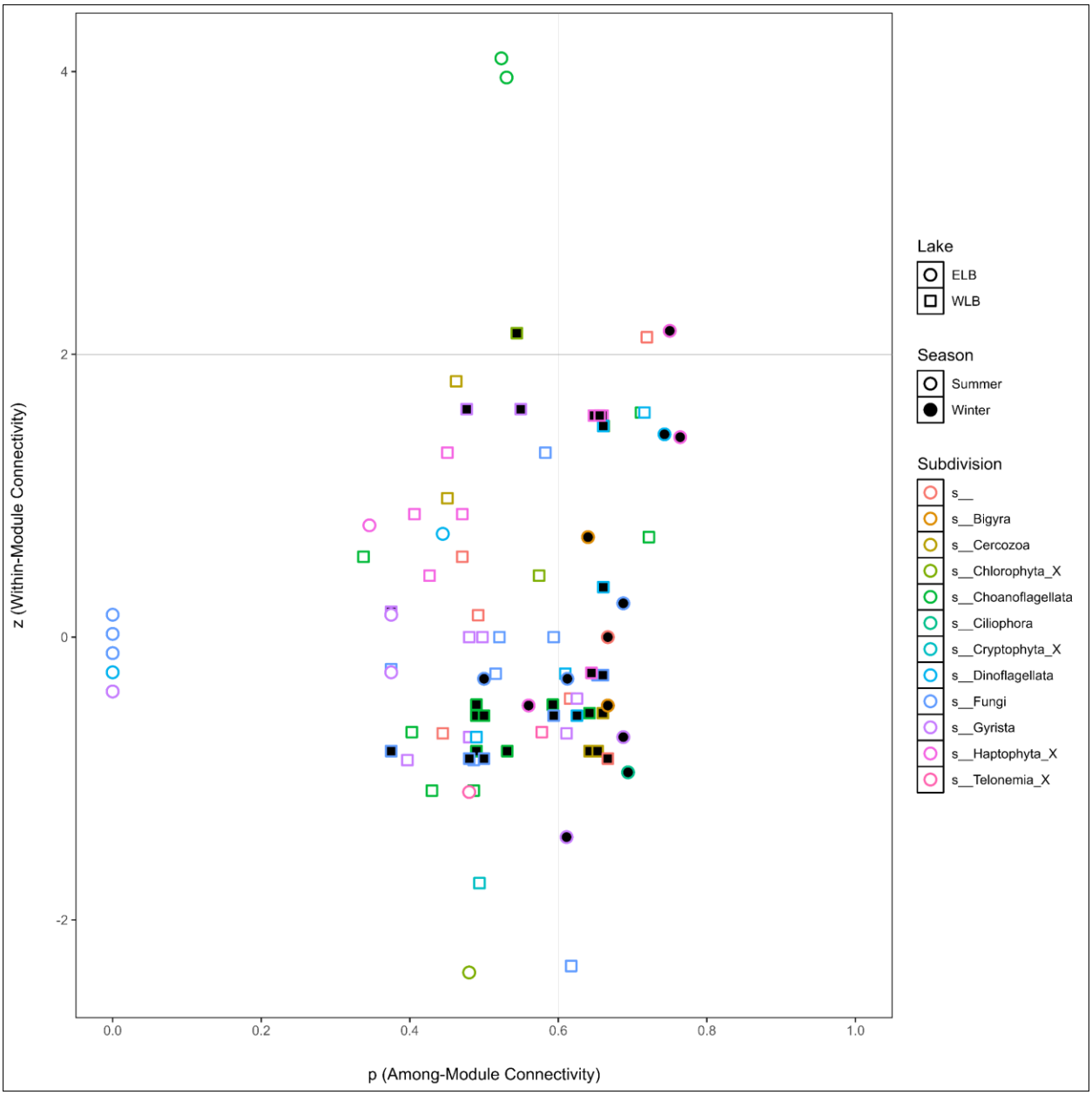

Figure 8 Within-Module (z) and Among-Module (p) connectivity scores or nodes in the ELB and WLB networks. Nodes are colored by their Subdivision, with filled shapes representing winter and open shapes summer. ELB is represented by circles and WLB by squares. Lines represent cutoff points for taxonomic roles, with the square on the bottom left representing peripheral nodes, the square on the bottom right representing connectors, the square on the top left module hubs, and the square on the top right network hubs. Overall, winter nodes show higher p score, meaning these networks are better connected to the full network. Chlorophyta and Haptophyta form module hubs and network hubs (respectively) in WLB and ELB (respectively) during the winter.

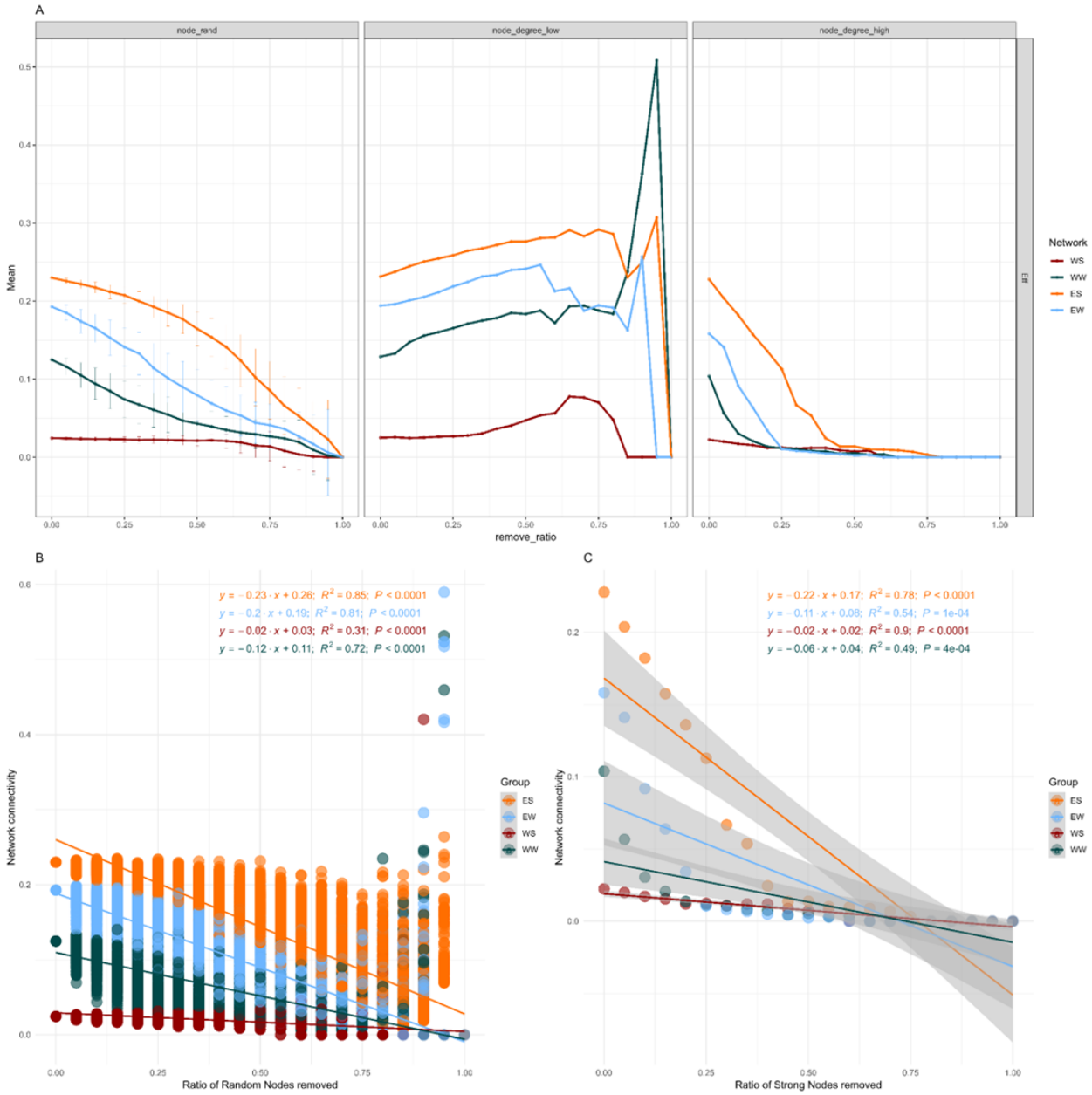

*Figure 9 Fitted regression models of Robustness. The top panel displays mean Global Efficiency on the Y-axis and the ratio of removed nodes on the bottom. The bottom panels show the fitted regression for each networks, with the left one showing the ratio of Random Nodes removed and the right one the ratio of strong (central) nodes removed. Slopes and R2 are displayed on the top-right. ELB shows higher initial robustness scores but show the greatest loss of Global Efficiency upon both random and strong node removal. Contrarily, WLB shows lower Global Efficiency but a less intense loss upon removal of strong nodes. ANCOVA (P<0.05) confirms that the Networks*node removal do significantly impact network efficiency, with all networks having statistically distinct slopes under the random model (p<2e10-16). Under the strong node removal, WS-ES (P<0.001), EW-WS (P=.03929), ES-WW (P<0.001) and EW-ES (P=.00571) significantly differed.*

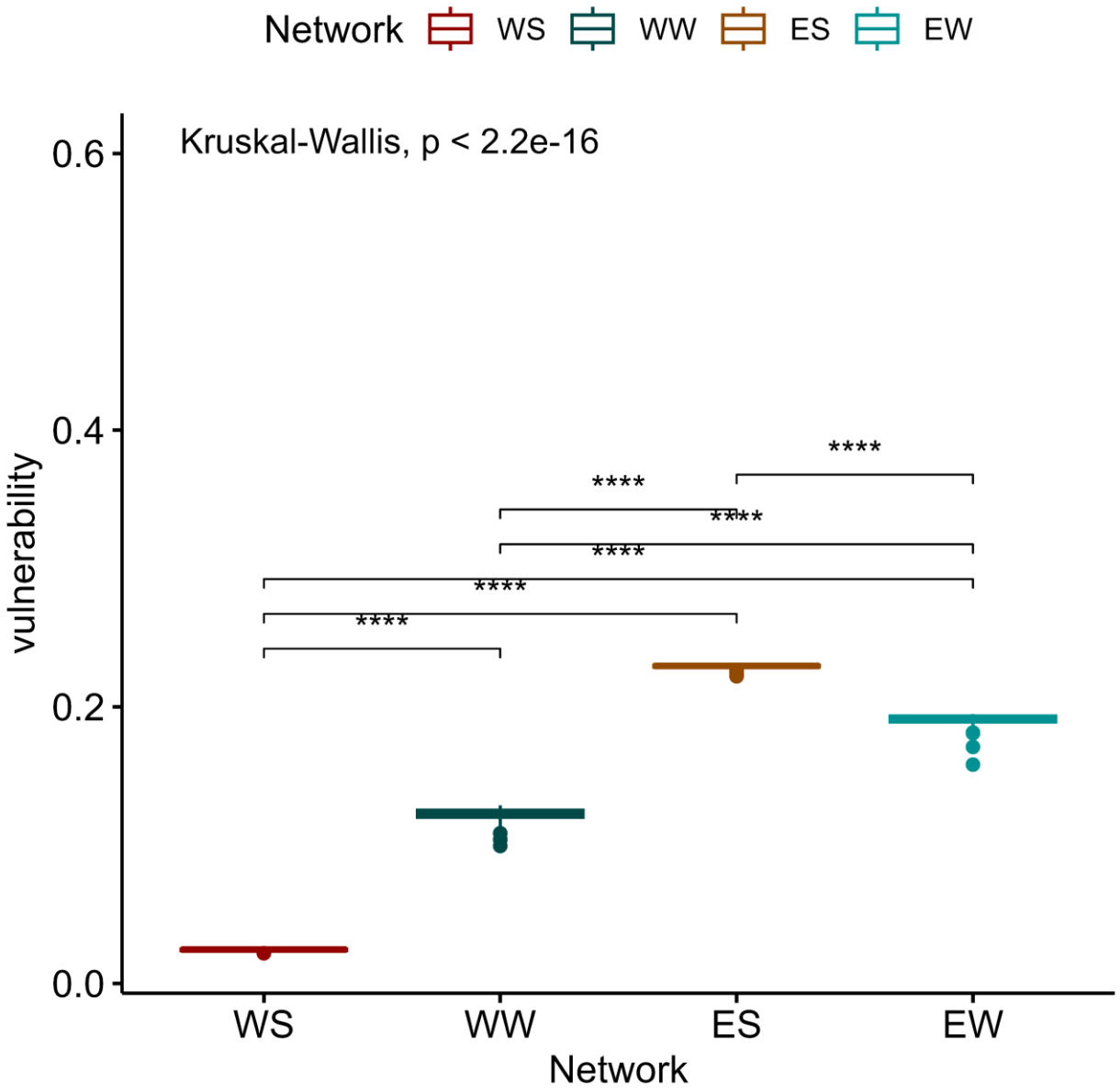

Figure 10 Mean Vulnerability score for each network. Kruskal-Wallis followed by a Wilcox-Test (P<0.05) was used to assess for significant differences between each network. All networks significantly differed in vulnerability, with ELB networks showing higher vulnerability than WLB networks.

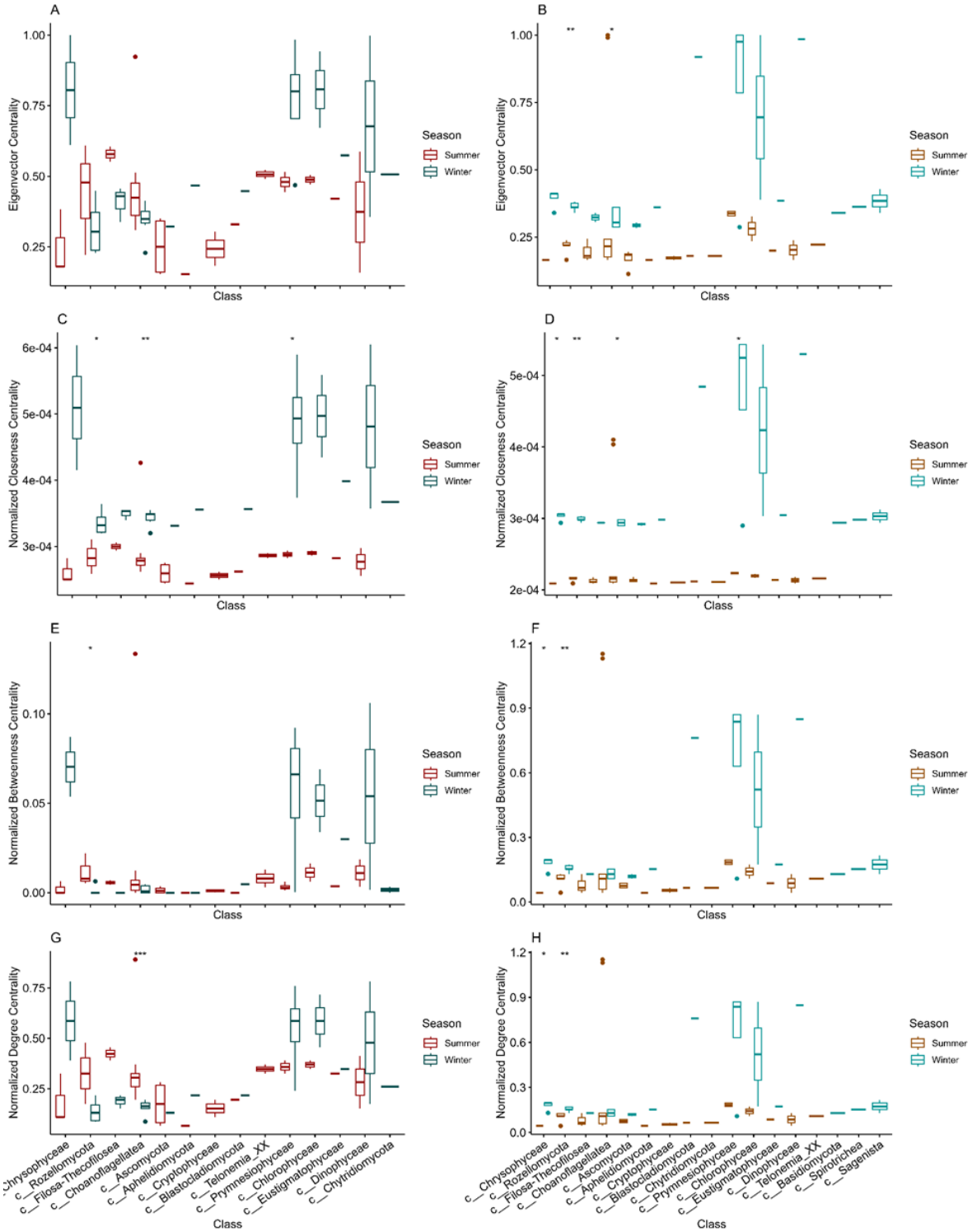

Figure 11 Average centrality scores for WLB (A, C, E, G) and ELB (B, D, F, H) by Class. Wilcox Test was performed to assess if winter and summer scores significantly differed for each taxa. Centrality Scores presented, from top to bottom, are Eigenvector Centrality (A, B), Normalized Closeness Centrality (C, D), Normalized Betweenness Centrality (E, F), and Normalized Degree Centrality (G, H). Rozellomycota shows significant differences in Closeness Centrality and Betweenness Centrality in both lakes, with Closeness Centrality being higher in the winter and Betweenness centrality being lake dependent. Prymnesiophyceae also shows consistently higher centrality scores, with their Eigenvector and Closeness Centrality being significantly higher in the winter across both lakes.

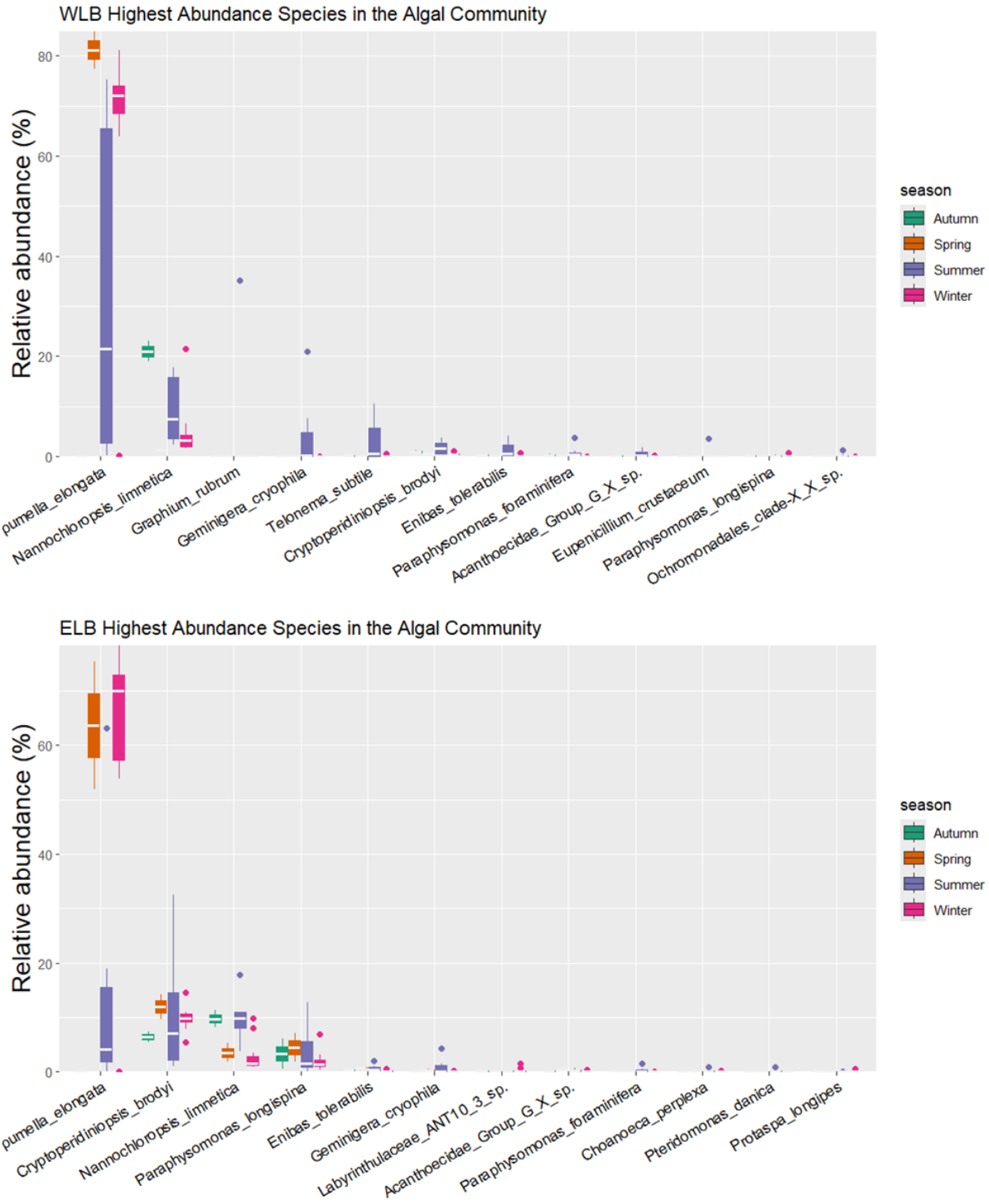

Figure 12 Relative abundance graphs displaying the highest abundance species in WLB (top) and ELB (bottom) within the algal community. Additionally, their relative abundance for each season is also displayed. Of those OTUs identified at species level, Spumella elongata and Nannochloropsis limnetica showed high abundance during the summer months. Further, Spumella elongata showed very high abundance in the winter and spring reaching values as high as 80% in WLB and 60% in ELB. Cryptoperidiniopsis brodyi also has higher abundance in ELB with generally higher abundance in spring and winter.

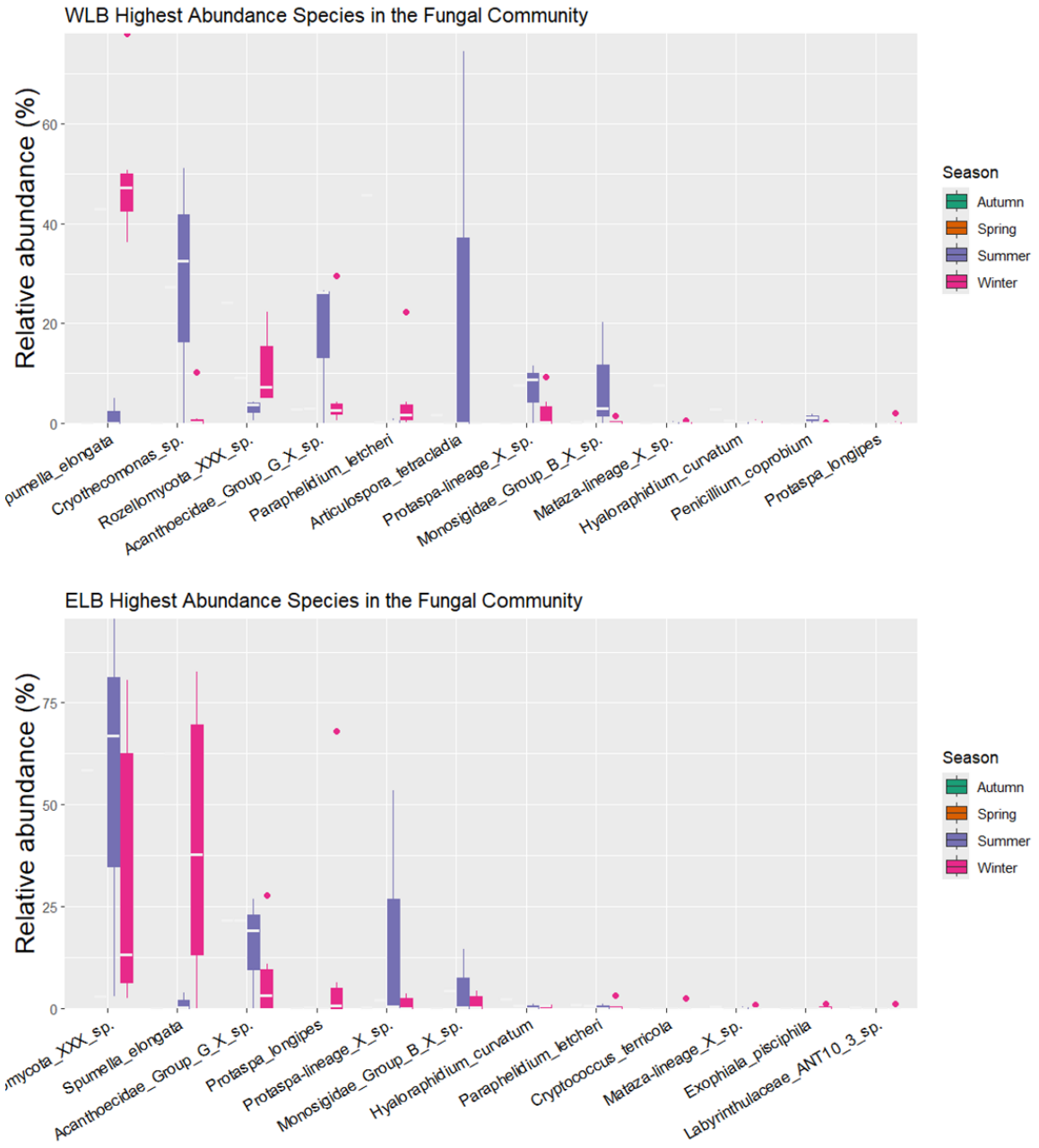

Figure 13 Relative abundance graphs of the displaying the highest abundance species in WLB (top) and ELB (bottom) within the fungal community. In both lakes, Spumella elongata is detected at high abundances with their relative abundance ranging between 40%-60% in the winter. In ELB, Rozellomycota shows high abundance both in the winter and summer, with higher abundances in the summer. Of those identified at species level, very few have abundances surpassing 10% meaning taxa only identified at higher levels are contributing greatly to these communities.

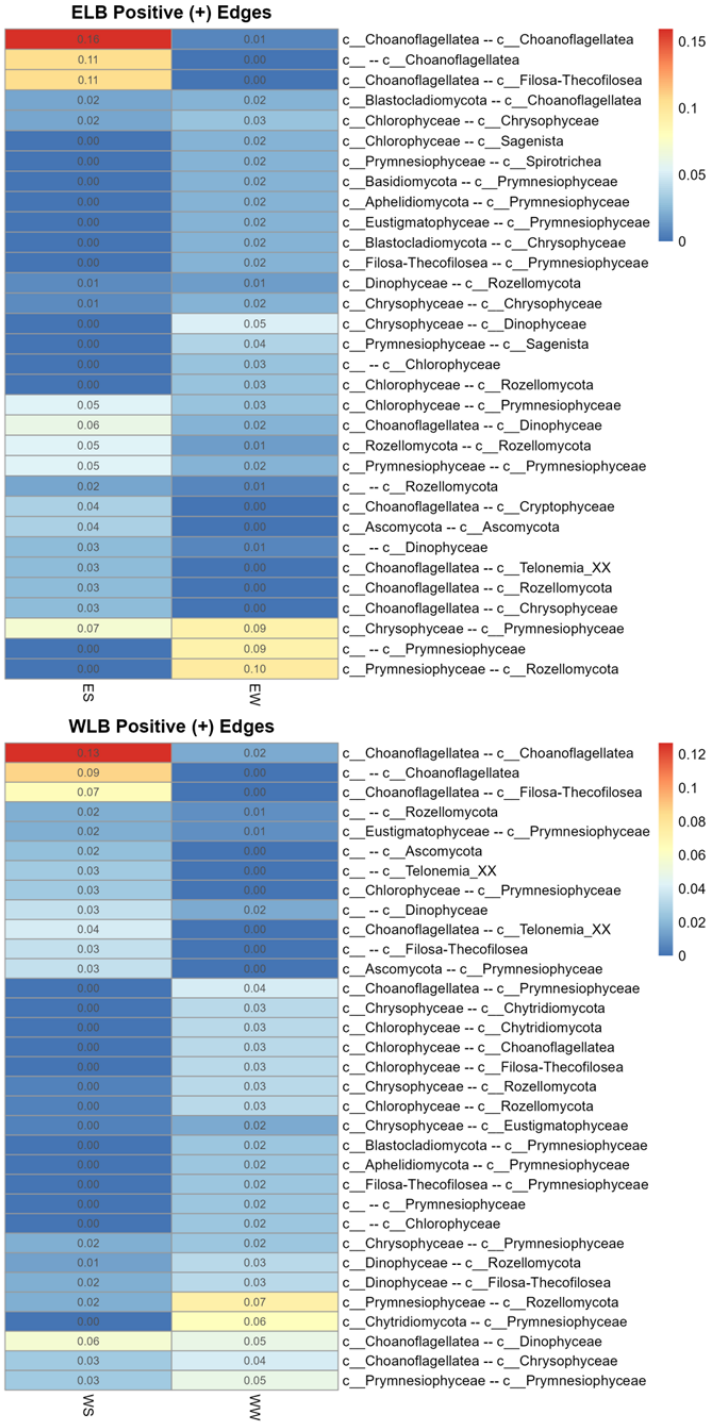

Figure 14 Heatmaps showing the normalized weights of positive edges between nodes organized at a class level. With ELB on top and WLB on the bottom. Columns are grouped by summer and winter networks respectively. Shifts in edges are observed between seasons in both lakes, with associations between pathotrophic taxa (Rozellomycota and Chytridiomycota) and algae (Chrysophyceae and Chlorophyceae) increaing in the winter. Further, mixotrophic taxa also show increased edges with these taxa in the winter.

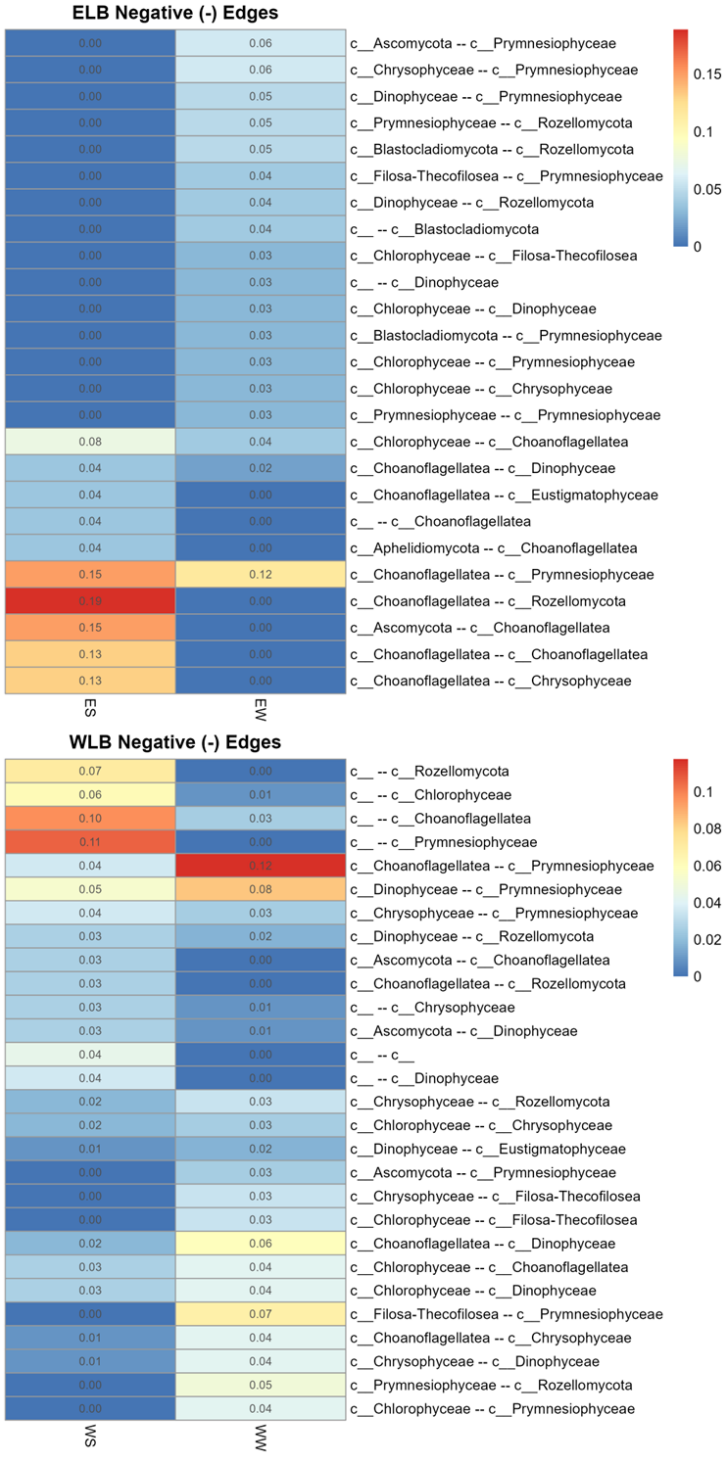

Figure 15 Heatmaps showing the normalized weights of negative edges between nodes organized at a class level. With ELB on top and WLB on the bottom. Overall, both lakes show differing negative edges. In ELB, Rozellomycota shows strong negative edges with Choanoflagellatea in the summer which are not observed in WLB. In WLB, Rozellomycota forms edges with Dinophyceae which includes predatory protists. These are stronger in the summer months.

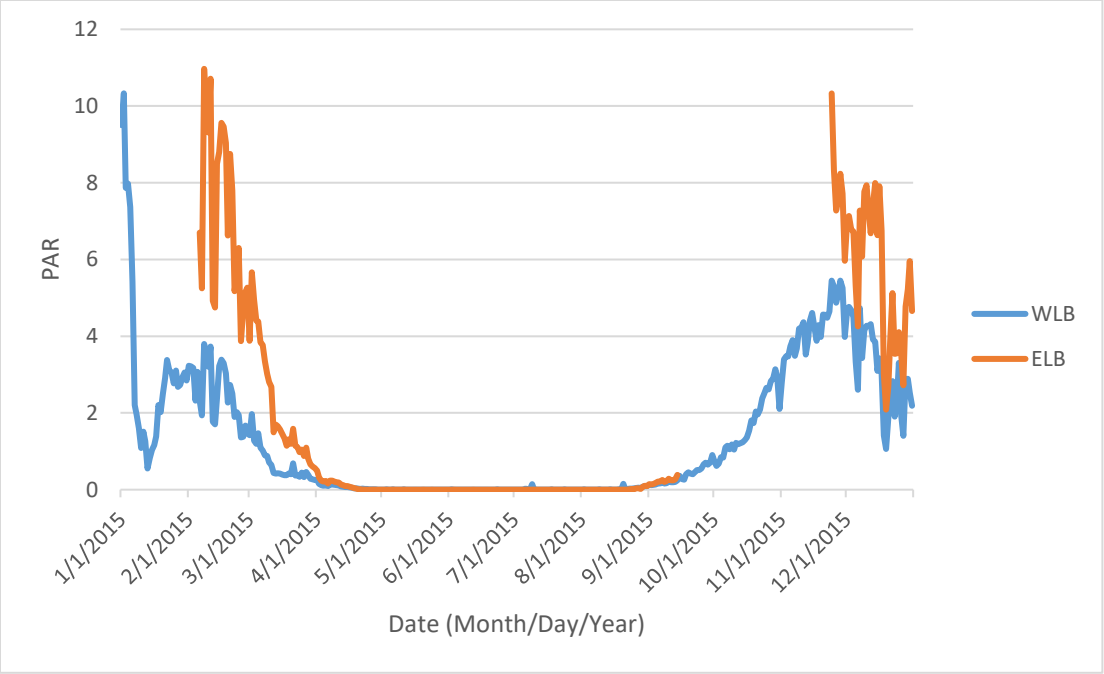

Figure 16 Underwater PAR from January 2015 to December 2015 for WLB (in blue) nd ELB (in orange). The data was collected by the blue boxes, which have PAR sensors anchored at around 5 meters in depth. Though not at the same depths as where our samples were collected, we can expect deeper depths to show similar trends. ELB shows higher par from mid February to April, reaching as high as 11 $\mu E*m^{-2}*s^{-1}$ in mid March and reaching 0 $\mu E*m^{-2}*s^{-1}$ in mid April. WLB only reaches a maximum of 4 $\mu E*m^{-2}*s^{-1}$ during February and also reaches 0 $\mu E*m^{-2}*s^{-1}$ at around mid April.

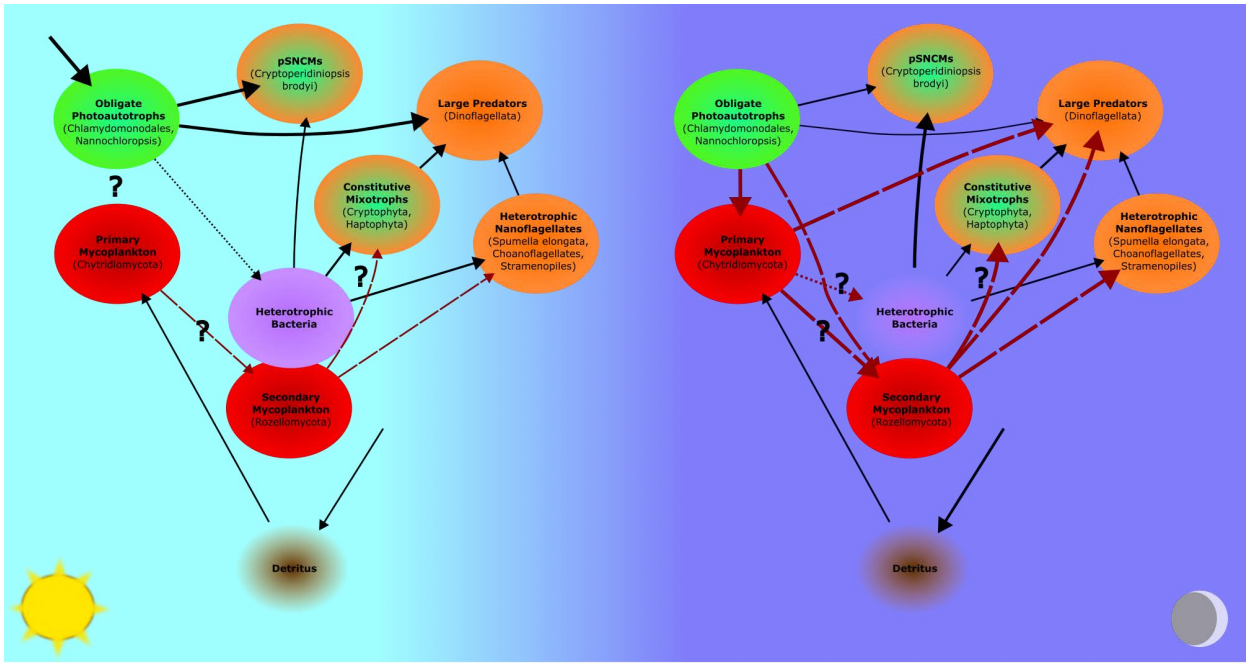

Figure 17. Hypothetical food web model of Lake Bonney, Antarctica during summer and winter. This was generated based on Morgan-kiss et al. 2024 and the co-occurrence networks generated in this study. Thickness of lines indicates the relative intensity of resource flow. Black lines indicate microbial loop resource flows and red lines indicate mycoloop mediated resource flows. During the summer, Obligate photoautotrophs provide DOM to heterotrophic bacteria, are predated more heavily on by large predators and pSNCMs, and predated a little bit by mycoplankton. Heterotrophic bacteria serve as prey for Constitutive mixotrophs, pSNCMs, heterotrophic nanflagellates, and predatory protists. Predatory protists can feed on all other organisms except for the pSNCMs. Primary mycoplankton, Chytridiomycota, likely serve as prey for large predators as well as Rozellomycota. Given the smaller size of Rozellomycota zoospores, these can be predated on by heterotrophic nanoflagellates, constitutive mixotrophs, and pSNCMs as well. In the onset of the polar night, obligate photoautotrophs undergo physiological responses which may make them more difficult to consume. On the other hand, these changes may make them more vulnerable to parasitic attachment. Thus, parasitic activity of Chytridiomycota and Rozellomycota would increase in the winter. We would expect predation of mycoplankton by large predators, heterotrophic nanoflagellates, pSNCMs, and mixotrophs to increase in the winter. Further, mycoplankton release DOM from their host which may serve to feed heterotrophic bacteria.

4) *Discussion*

Revisiting the aim of this study

Fungi are important contributors to global nutrient and carbon cycling: this is particularly appreciated in soil ecosystems where soil fungi play key roles in the plant root system and the degradation cycle of complex organic matter. A number of fungal groups with diverse ecological roles in aquatic ecosystems have been found to play a major part in carbon cycling within these systems. The mycoloop is a concept describing the role of parasitic fungi in transferring nutrients from large, inedible algae via infection, allowing for enhanced access to recalcitrant material to the rest of the food web which bypass the microbial loop. Flagellated fungi belonging to Rozellomycota and Chytridiomycota phyla, are recognized as important members of aquatic food webs (Chen, et al. 2024). These parasites feed on nutrient poor hosts, while their zoospores are rich in nitrogen and polyunsaturated fats (Gleason et al. 2012; 2008), translating to an "upgrade" prey quality. Patterns in the abundance of these parasites is tied directly to the availability of hosts (Van den Wyngaert et al. 2022), and as such are expected to follow similar trends in abundance to its host. Phytoplankton growth and biomass levels exhibit predictable seasonal trends, owing to the availability of nutrients and light.

In polar systems where summers and winters are characterized periods of prolonged light and prolonged darkness (Johnsen et al. 2020; Wietz et al. 2021), phytoplankton communities also show strong seasonal trends (Patriarche et al. 2021). In addition, a previous report observed relatively high levels of parasitic fungal taxa in the Antarctic McMurdo Dry Valley lakes (Rojas-Jimenez et al. 2017). Given these previous results, we predict that the influences of the mycoloop could have a significant role in the MDV lakes. As such, this study sought to dissect the host-parasite dynamics between mycoplankton and their algal hosts in one of the well-studied MDV lakes, Lake Bonney. Our goals were to (i) describe the fungal diversity in Lake Bonney, (ii) characterize seasonal trends in fungal and algal taxa, and (iii) develop networks to understand how microbial interactions change during summer and winter. Overall, this study will advance understanding of the importance of these fungi and their dynamics across the seasons, including potential roles for the mycoloop in the MDV food web.

The Algal Communities show distinct seasonal trends.

The polar night presents a unique time of year in which the loss of light causes the cessation of photosynthetic activity for several months. Within polar marine systems and other aquatic systems open to wind driven mixing, winter communities are defined by higher activity and growth of chemoautotrophic bacteria, as well as nutrient regeneration (Wietz et al. 2021) which comes from deeper in the water column. However, how microbial communities within hydrologically stable systems such as the MDV ice covered lakes respond to polar night is not as well understood. The MDV lake food web are almost entirely dependent on autochthonous carbon from its phytoplankton, with summer streams inputting low quantities of DOM (Fountain et al. 2014; Green and Lyons 2009). As such, we would expect that the microbial food web would be significantly impacted by the loss of autochthonous carbon production during the winter months. A recent study based on spectral chl-a fluorescence reported that phytoplankton were unexpectedly active during the polar night, with chl-a levels, repsenting a proxy for algal biomass, remaining steady in ELB and even increasing in WLB through the winter. This report correlated the winter rinse in Chl-a with the 'mixed' community of algae (Patriarche et al. 2021). Another study using 16S rRNA and 18S rRNA amplicon sequencing in water samples from West Lobe Bonney reported significant changes in communities during the shift from summer to winter. Based on sequence data, the authors suggested that community function shifted towards mixotrophy, chemolithoautotrophy, and heterotrophy (Vick-Majors et al. 2014). Vick-Majors et al suggested that mixotrophic genera such as *Ochromonas* would make up a major part of the community. However, this study was very limited in sampling, with only one sample collected each during summer and autumn.

In the current study, we expanded on previous studies by providing seasonally-intensive sampling data. Summer communities of algae detected in this study compared well to previous studies on the eukaryal communities present in lake Bonney (Lee et al. 2024; Morgan-Kiss et al. 2024) with the ALPS samples, which were anchored around 15.5 meters in depth, reflecting the higher abundance of Haptophyta (Prymnesiophyceae), Cryptophyceae, and high abundances of Stramenopiles (Chrysophyceae, Eustigmatophyceae). The algal community detected by the V9 primer set showed that Chrysophyceae differ significantly in abundance in the winter (Figure 3A, B, 5A, D). This

increase in abundance is due largely to *Spumella elongata* (Figure 12), a colorless predatory chrysophyte (Grossmann et al. 2016), and members of Ochromonodales which includes *Ochromonas.* Clear differences in species richness (Figure 2A) and taxonomic composition (Figure 4A, 4B) were observed between summer and winter in both lakes. As suggested by Patriarch et al. (Patriarche et al. 2021), Isochrysidaceae (Prymnesiophyceae) is an abundant member of the winter phytoplankton community in both lakes along with Ochromonodaceae in both lakes. This group of mixotrophs are likely relying on bacterial prey for their continued survival during this season. Further, Dinophyceae also maintains a high abundance in the winter.

Dinoflagellates are at the top of the food web in the McMurdo Dry valleys (Rachael M. Morgan-Kiss et al. 2024) preying on Heterotrophic Nanoflagellates, Mixotrophs, Bacteria, and Photoautotrophs. The most common Dinophyceae read was attributed to *Cryptoperidiniopsis brodyi*, a Plastidic Non-Constitutive Mixotroph (pSNCM). pSNCM's acquire chloroplasts by the consumption of specific prey and are thus expected to be competitive when this prey is in high abundance and light is available (Mitra et al. 2023). Sequences of this mixotroph show higher abundances in the autumn and early summer of WLB but not ELB. Interestingly, WLB shows a higher abundance than ELB, with Dinophyceae, largely *Cryptoperidiniopsis brodyi*, increasing in abundance in May in WLB and July in ELB. Given the stable hydrology of these lakes, light is expected to be the major drivers of the phytoplankton community. Available light in WLB and ELB differs, with ELB showing higher light availability up until the sunset around April (Figure 16). It is possible that mixotrophs become more competitive earlier in the season in WLB. Previous studies have also found that, during other sampling years, available light can drop below 0 umol photons m^{-2} s^{-1} as early as January in WLB (Patriarche et al. 2021). This further reinforces the idea that light is the main factor in shaping winter phytoplankton dynamics.

The fungal community shows high abundance of pathotrophic fungi in the winter which may be linked to enhanced mycoloop activity

The diversity and function of fungi within the McMurdo Dry Valley Lakes has been largely neglected, with only one other study characterizing the communities present in the MDV (Rojas-Jimenez et al. 2017) and none to our knowledge characterizing winter

communities. As such, this study is the first describing how the composition of the fungal community shifts between austral summer and polar night. A study by Rojas-Jimenez et al found that the McMurdo Dry Valley Lakes are dominated by Chytridiomycota and Cryptomycota (Rozellomycota) (Rojas-Jimenez et al. 2017). To that end, we selected the same V7-V8 primer set used in their study so we could compare seasonal trends to existing studies. Based on the results of our study, we were able to confirm the high abundance of basal lineage fungi in Lake Bonney. The basal lineages of Rozellomycota, Aphelidiomycota, and Chytridiomycota all have the capacity to parasitize phytoplankton, meaning that they have the capacity to impact host community structure, biogeochemical cycles, and even host evolution (Ilicic and Grossart 2022). Interestingly, the three groups differ in their patterns of abundance. Aphelidiomycota and Rozellomycota both show high abundances in the late summer and autumn, with Chytridiomycota and Rozellomycota showing high abundances in the winter months. Thus, it appears that these parasites may be impacting the algal community year-round. Contrarily to Rojas-Jimenez et al., our study found that WLB shows a higher abundance of Chytridiomycota, while ELB is dominated by Rozellomycota (Figure 1C, 1D). Given that the previous study was performed on data collected in the summers of 2011-2012, it is possible that the communities may have shifted due to ongoing lake level rise (Gooseff et al. 2017) or that the 2015-2016 sampling year is not representative of the community. Resequencing the full archived ALPS samples may provide a clearer picture as to typical fungal community trends. Regardless, the summer communities detected in both ELB and WLB differ dramatically in the identity of present taxa. However, no significant differences in species richness and diversity were detected between the two lobes and only WLB showed significant differences in taxonomic composition (Figure 3). This difference in taxonomic composition is strongly driven by Chytridiomycota and Rozellomycota which significantly increase in abundance in the winter (Figure 2D, 4D). It is unclear why this difference in taxonomic composition is only observed in WLB. Regardless, it appears that fungal community diversity remains stable year-round, with the dominant taxa being lake dependent.

The characterization of ELB's summer community remains limited due to a number of summer samples being filtered out during sample processing. As such, interpretations of how their community differs between summer and winter are limited. Regardless, we characterize

the community here. East Lobe Bonney shows a high abundance of Rozellomycota from February to August, which peaks both in February and between June and July. High abundance of Chrysophyceae (*Spumella elongata*) (Figure 13) is also detected during the winter in this dataset, reinforcing the importance of this predator in the winter. Further, decreases in Rozellomycota abundances were followed by increases in Choanoflagellatea, which includes heterotrophic nanoflagellates (HNFs). Both of these taxa may present potential predators of Rozellid zoospores. HNFs typically predate on bacteria. However, any protists smaller than them (2-20 μM) (Richter and Nitsche 2017; Schiwitza et al. 2019) may also serve as prey. Rozellomycota zoospores can be amoeboid or flagellated and generally fall between 1.2-2.0 μM in size (Letcher and Powell 2018) meaning they can be predated on by these bacterivorous HNFs. Interestingly, another predator, *Fisculla terrestis* (Thecofilosea, Cercozoa) displayed selective predation towards fungal cells (Dumack et al. 2019). Thus, it may be possible that other protist predators could display similar selectivity. It is unclear what taxa may serve as Rozellomycota's main prey source. Little is known of their ecology, and Rozellomycota has a wide range of hosts including Amoeba (Corsaro et al. 2014), Oomycota, Blastocladiomycota, Chytridiomycota, Monoblepharidomycota, Basidiomycota, and green algae from Charophyceae (Letcher et al. 2018), increases in winter abundance suggest that these mycoplankton have easier access to their prey.

WLB shows similar trends in abundance for its dominant mycoplankton, Chytridiomycota. Chytridiomycota were detected in low abundance from February to April, with their abundance then increasing and remaining high from April to August. Rozellomycota was also detected year-round in WLB at low abundances, increasing in abundance during the late winter. Increases in Filosa-Thecofilosea, a testate amoeba, and Choanoflagalletea follow from September to December. These, along with *Spumella elongata* may present important predators for both Chytridiomycota and Rozellomycota. Differential abundance confirmed that Chytridiomycota, Rozellomycota, and Chrysophyceae are significantly more abundant in the winter. A study performed in a dimictic, mesotrophic, temperate lake found that mycoplankton parasites increase in abundance following blooms in their host, generally occurring at peaks of productivity (Van den Wyngaert et al. 2022) during the spring and summer. As such, we would expect mycoplankton abundances to lag

behind potential hosts. However, this is not observed in Lake Bonney. Peaks in the abundance of Chytridiomycota and Rozellomycota do not seem to follow any peak in potential phytoplankton prey. As such, the main biological drivers of their increased abundance cannot be explained by the abundance patterns of potential hosts alone. Taken together, our findings of several predatory fungi exhibiting increasing abundance in the winter further suggests the importance of heterotrophy in the winter. More importantly, our data implicate a larger role for the Mycoloop in the winter than in the summer months. With the onset of polar night, photosynthetic organisms such as Chlorophytes undergo various physiological changes (Morgan-Kiss et al. 2016), maintaining their photosynthetic apparatus through the winter, albeit shut down. Further, the accumulation of lipids and formation of spores are other strategies which these algae may employ (Johnsen et al. 2020). This may impact the ability for predators to access this prey as these changes may impact their size or overall edibility. Further, some green algae with facultative multicellularity have been shown to induce it under predation pressure (Herron et al. 2019; Cornwallis et al. 2023) which is expected to be higher in the winter. By parasitizing on these large, inedible phytoplankton in the winter, Chytrids and Rozellids would make nutrients and carbon more available to other predators such as nanoflagellates and dinoflagellates (Ilicic and Grossart 2022; Grossart et al. 2019; Thongthaisong et al. 2022). Specifically, given the low Carbon:Nutrient ratios of zoospores (Ilicic and Grossart 2022), these make for more nutritious prey rich in lipids which are could be quite desirable in phosphorous limited systems (Sime - Ngando 2012). Further, Rozellomycota are also able to prey on Chytrids, which adds another layer of complexity to the food web and enhances nutrient turnover (Gleason et al. 2012). Rozellomycota zoospores are even more edible than Chytrid zoospores due to their smaller size (less than 5 μm) (Letcher and Powell 2018). As such, Rozellomycota likely enhances carbon flow in the winter as both large bacterivores and protist predators can consume these.

Rozellomycota is significantly more central to winter networks, providing further evidence for a role for the Mycoloop during the winter

Within ecological co-occurrence networks, taxa with higher betweenness centrality and closeness centrality are expected to be more important to the maintenance of networks

structure by enhancing resource spread (Oña et al. 2025). Within the context of a food web, species with higher closeness centrality are associating more with others, with these associations potentially denoting shared prey, resources, or predation pressures (Estrada 2007; Martínez Rendón et al. 2025). In both lakes, winter networks show that Rozellomycota and Chytridiomycota increase in Closeness Centrality (Figure 11) with Rozellomycota showing significant differences. This increase in Closeness Centrality indicate that these taxa are becoming more important for resource flow and network structure, likely allowing greater resource flow from algal prey to predatory protists and bacterivores. Effectively, Rozellomycota forms stronger connections with potential green algae prey (Chlamydomonadales), predatory protists (*Spumella elongata*), and Mixotrophs (*Cryptoperidiniopsis brodyi* and Prymnesiophyceae) during the winter months. Chytridiomycota also shares connections to Chlamydomonadales and Prymnesiophyceae but also shows connections to Mychonastes, which may represent a mixotrophic green algae. Thus, it seems that these mycoplankton exert greater influence on the connectivity of the communities in the winter likely linking inedible green algae with larger predatory protists.

Betweenness centrality can be an important network metric for interpreting how important noeds are for the flow of resources (Oña et al. 2025). Interestingly, betweenness centrality, showed some interesting lake-dependent trends. In ELB, Rozellomycota exhibited higher betweenness centrality in the summer. In WLB, Rozellomycota displayed higher betweenness centrality in the winter. Chytridiomycota may follow similar seasonal patterns as Rozellomycota, but their absence from the summer networks makes this assessment difficult (Figure 10). High betweenness is an additional metric which can provide important ecological information, indicating that a node connects more nodes in the network together thus acting as a bridge in the networks. It has been shown that, under increased predation, betweenness centrality within networks increases (Martínez Rendón et al. 2025). Given that Rozellomycota can serve prey to predatory protists, this higher betweenness centrality may indicate that they are linking inedible prey with larger predators . In ELB, Rozellomycota forms a strong association with other Rozellomycota as well as Choanoflagellates. It is unknown if Rozellids are capable of parasitizing other Rozellelids, but it is possible that Choanoflagellates are feeding on these in the summer as Rozellids have zoospores small enough to be ingested by these bacterivores, thus explaining their higher

Betweenness Centrality. In WLB, the higher Betweenness Centrality is most likely linked to the increased parasitism of Chlamydomonas. Thus, it seems that predation pressure on this mycoplankton shifts seasonally, with choanoflagellates likely preying on them directly in the summer while larger predatory protists feed more readily on them in the winter.

Lake Bonney Mycoloop: potential interactions, seasonal trends, and unresolved questions

Co-occurrence networks allow for the detection of co-occurrence patterns which, in the context of ecological networks, can represent associations due to shared prey and or resources, direct associations such as predation, competition, and mutualisms, or indirect associations which result from indirect effects from a third-party (Freilich et al. 2018; Martínez Rendón et al. 2025; Lee et al. 2022). Though these inferred associations alone can not confirm interactions, the nature of the taxa (for eg. the trophic mode) can help in identifying potential interactions (Martínez Rendón et al. 2025). When observing the edges formed between the mycoplankton and the rest of the network, seasonal differences were observed (Figure 14, 15). Across both lobes of Lake Bonney, Rozellomycota formed stronger connections with *Spumella elongata*, (Chrysophyte, Predator), Chlamydomonadales (Chlorophyceae, Phototroph), and *Cryptoperidiniopsis brodyi* (Dinophyceae, pSNCM) in the winter. In the summer, Rozellomycota formed stronger connections with Choanoflagelletea (Bacterivore) across both lakes. Additionally, Rozellomycota formed strong edges with *Hyaloraphidium curvatum* (Blastocladiomycota, saprotroph) in the summer only in ELB. Though no direct associations were detected between Rozellomycota and Chytridiomycota, it is expected that Rozellomycota would act as a hyperparasite of Chytridiomycota based on current knowledge of their ecology (Van den Wyngaert et al. 2022; Letcher and Powell 2018). Chytridiomycota showed more limited connections shared across both lakes, only forming edges between Chlamydomonas (Chlorophyta, Phototroph), Mychonastes (Chlorophyta, Mixotroph), and Prymnesiophyceae (Isochrysidales, Mixotroph) in the winter. Given these observations, differences in centrality between seasons, patterns of abundance, and strong edges observed in the networks, we can begin to map out how the mycoloop interfaces with the foodweb described in Morgan-Kiss 2024 (Figure 17).

The major mycoplankton groups, Chytridiomycota and Rozellomycota, show limited association with phytoplankton in the summer months as well as lower closeness centrality.

Given that no green algae blooms coincide with the increased abundance of these parasites in the winter, the increased abundance of these parasites in the winter may be linked to shifts in detritus availability or seasonal-dependent shifts in host physiology. The ability of the zoospores to attach to its host is dependent on physical conditions such as temperature and host resistance (Scholz et al. 2017). It is possible that hosts are better able to mount chemical and physical defenses against zoospores in the summer, with such methods including anti-fungal production or Mucilage production which is primarily used as a way of mitigating ROS and Light damage (Busch and and Hess 2022; Van Den Wyngaert et al. 2022) but has been found to limit chytrid zoospore attachment as well (Van Den Wyngaert et al. 2022). Further, higher species richness in the summer may indicate higher intraspecific diversity within Chlamydomonadales. Studies have seen that chytrid infection rates are lower in genetically heterogeneous host mixtures (Agha et al. 2018), likely because Chytrids require host specific cell surface features to attach to before initiating infection. Presumably, this may also limit Rozellomycota infection on green algae. Due to lower abundances of chytrids, the activity of the mycoloop is likely less in the summer, with saprotrophic chytrids being more abundant. Further, Rozellomycota specific to chytrids may also be in lower abundance unless other prey, such as Blastocladiomycota, is present. Predation of Mycoplankton zoospores may thus be limited to Heterotrophic Nanoflagellates (HNFS) such as Choanoflagellates feeding on Rozellomycota zoospores in the summer.

As Polar night sets in, the phytoplankton community shifts in response to changing availability of light. Mixotrophic taxa can remain active in the winter, increasing or maintaining their abundance, while phototrophic taxa enter dormant states which may be less resistant to attachment by zoospores. Because of these changes in the food web, the activity of these mycoplankton increased during the night. This is supported by the significantly higher abundances, higher closeness centrality, and the edges that these taxa form in the winter. During the winter, mixotrophs feeding on bacteria may also feed on Rozellomycota zoospores. Based on the network edges, it seems that large predators such as Dinoflagellates and pSNCM's such as *Cryptoperidiniopsis brodyi* more readily feed on these in the winter. Further, we would expect chytrid zoospores would also be potential prey for these larger predators. When infection rates are high, and thus competition between chytrids, it has been observed that zoospore size decreases to allow for shorter generation times which would

allow them to maximize their transmission (Agha et al. 2018). These smaller chytrid zoospores would thus be more accessible to predators.

Network robustness and vulnerability differ seasonally and by lake, implying differing community stability

Analysis of network stability (robustness and vulnerability) revealed that ELB showed greater global efficiency than WLB (Figure 9A). This is expected given that ELB networks showed overall higher centralization and lower clustering coefficients (Table 4). In an ecological context, more centralized networks can exchange resources more effectively as there are nodes acting as central hubs. Effectively, ELB networks show Choanoflagellate OTU's act as module hubs in the summer, with Haptophyta acting as a network hub in the winter (Figure 8). Fewer hubs are observed in WLB, with only Choanoflagellatea and Cercozoa (represented by s__) being module and network hubs respectively (Figure 8). On the other hand, this higher centralization may also explain why the ELB networks are less stable.

We hypothesized that winter networks would show greater robustness given the decreases in modularity observed in the networks. Though this was the case for ELB, WLB showed no significant differences in its network robustness, with its summer network being more robust (Figure 8C, Table 6). Network vulnerability mirrors these trends as well (Figure 9). This was unexpected, and it is unclear what features of the networks, and by extension microbial communities, lead to these differences in stability. It Is interesting that the winter networks do not differ from each other in robustness. This suggests that winter conditions, and the resulting community, reach a similar stable state regardless of the robustness of their summer community. However, this is purely speculative and would require in-depth analysis beyond the scope of this study to explore. A study on the robustness of networks across the McMurdo Dry Valley lakes may help answer what physical, chemical, and biological features most impact the stability of these communities.

Conclusions and Future Directions

The major goals of this study were to 1) characterize the fungal diversity present in Lake Bonney in the MDV which may have been missed by the V9 primer set used across several

studies, 2) characterize how the protist communities identified by both primer sets shifted between polar day and night and explore which taxa define each season, and 3) using a co-occurrence network approach, assess how mycoplankton interactions with their hosts and predators may be changing across seasonal scales. We hypothesized that the V7-V8 primer set would better target the basal lineage fungi Rozellomycota and Chytridiomycota as these primers have been used in prior studies. Further, we hypothesized that the communities identified by the primer sets would show significant differences in diversity and taxonomic composition as light is a major factor in shaping these communities. Lastly, we hypothesized that mycoplankton would increase in centrality in the winter, reflecting increased parasitic activity and higher predation of their zoospores by predatory protists.

Of all the primer sets, the ITS yielded the lowest number of reads & identified sequences. The Dataset was able to mirror some of the abundances of Rozellomycota & Chytridiomycota observed in the V7-V8 primer set. However, a vast majority (50-90%) were uncharacterized at a class level. This may be due to high abundances of rare, uncharacterized taxa; the more likely reason may be due to the limitations of the selected region and sequencing technique. The 5.8S-ITS2 region which this primer set targeted, and works well with basal lineage fungi, is highly variable in length between fungal clades with some ITS regions ranging from 14 bp to 730 bp (S. Chen et al. 2010). Given that these samples were processed in tandem with the 18S using a nexterra kit, it is possible that the amplicon sequencing approach failed to accurately capture these sequences as the Miseq sequencer is only able to accurately read sequences up to 600 bp in length. As such, many of these uncharacterized taxa may belong to taxa with ITS2 regions falling out of the size range of what our sequencing methods could effectively identify. Future use of this primer set may require different techniques to account for the variability in sequence length. Methods such as ribosomal tandem repeat barcoding (Wurzbacher et al. 2019), which involves coupling primers to cover all ribosomal markers, would better identify the fungal reads detected with these primers.

Ultimately, we confirmed that the algal community showed distinct communities with lower species richness in the winter, likely due to the dominance of select mixotrophic and predatory taxa within Chrysophyceae, such as *Spumella elongata*, and dinoflagellates such as *Cryptoperidiniopsis brodyi*. The fungal community did show higher abundances of

Rozellomycota and Chytridiomycota but was also able to identify other heterotrophic protists which were not detected in high abundance with the V9 primers. This community did not show significant differences in species diversity, suggesting that heterotrophic protist communities (including pathotrophs) are not as deeply impacted by the onset of polar night due to the diversity of their trophic modes. It is possible that, if we only considered the fungi, that we would have observed significant seasonal differences as these would be more limited by the availability of prey. The ITS primer set, which seemed to capture mostly fungi, did not show significant seasonal differences either. However, the issues presented by this primer set limits our ability to confidently answer this question. Additionally, West Lobe Bonney, which is dominated by Chytridiomycota, did show that pathotrophic and predatory taxa are significantly more abundant in the polar night. The same trend was not observed in East Lobe Bonney, as Rozellomycota showed higher abundance in February as well as the polar night. It is possible that missing data led to this problem. Further, our analysis is also limited by our sampling period which only covered the 2015-2016 sampling season. It is possible that we missed patterns that occur on multiyear scales, or, more likely, that we missed shifts in the community which may be occurring at a monthly scale. Studies performed in other arctic systems (Wietz et al. 2021) and in the MDV Lakes (Vick-Majors et al. 2014) show that eukaryal communities did show significant differences across all seasons. Though this was not observed in our data, the algal community did have high differences in species richness and diversity. More samples from other sampling years may help clarify these trends and allow us to perform more rigorous analysis for the autumn and winter seasons. Regardless, it seems that conditions in the polar night are better able to support pathotrophic taxa. Likely the physiological shifts purely phototrophic algae such as Chlamydomonodales undergo facilitate zoospore attachment and resulting infection. Further, high infection rates may increase competition between these parasites thus favoring smaller zoospores which can then be preyed upon by a wider range of mixotrophic and heterotrophic protists.

The co-occurrence networks confirm predatory and mixotrophic taxa, Chiefly those in the Class Chrysophyceae, Prymnesiophyceae, and Dinophyceae are amongst the most central to the winter communities. These predators, along with the dormant Chlorophytes, are important in determining the shape and function of the community. Though less important, Rozellomycota and Chytridiomycota do show increases in their Closeness Centrality which

indicates that these taxa are enhancing connectivity by providing shorter paths between taxa. In this case, these taxa provide alternate means of accessing carbon from normally inedible phytoplankton. The degree by which they enhance the flow of resources, their Betweenness Centrality, seems to be lake specific and more closely related to when these mycoplankton see their highest abundances.

Lastly, the co-occurrence networks allowed us to generate a hypothetical food web. This food web describes how the protist community shifts upon the onset of polar night, with the winter communities showing enhanced predation of mycoplankton zoospores, chiefly Rozellomycota, by predatory protists. Though plausible based on the data presented in this and past studies of mycoplankton ecology (Kagami, Miki et al. 2014; Chen et al. 2024; Van den Wyngaert et al. 2022), the co-occurrence network approach alone cannot confirm that these interactions are indeed occurring. Given more limited knowledge of Rozellomycota ecology and host range, it is difficult to differentiate which associations may indicate a shared host, parasitism, predation, or indirect co-occurrence. Further, comparing positive/negative edges can be misleading as complex interactions impact these associations (Boyse et al. 2025).For example, predation can be indicated by co-occurrence (+) in the case of predators successfully tracking down prey or by co-exclusion (-) in the case of prey avoiding a predator (Boyse et al. 2025). Given that Chytrids and Rozellids do display chemotaxis (Agha et al. 2018; Gleason et al. 2012), we would expect that co-occurrences would indicate parasitic interactions if the host is compatible which is observed with the edges between these parasites and Chlamydomonadales. Further, chosen network methods affect false positive rates (Martínez Rendón et al. 2025). Our decision to use the SparCC algorithm for network generation was done with the goal of minimizing spurious associations (Friedman and Alm 2012) and thus we feel confident that our study captures true associations. Further, the inclusion of trophic mode increases our confidence of our interpretations further. However, our data is limited to only 1 year of study. This means that we may be missing variations which occur by year or cycle which span over many seasons, such as what we may be observing in ELB.

Regardless, this hypothetical food web is meant to be used as a springboard to explore relevant ecological questions. By providing taxa of interest to test interactions between mycoplankton and hosts, we can begin to generate questions related to the ecology of these

basal lineage fungi. Future experiments exploring how shifts in Chlamydomonodales physiology between summer and winter impact their susceptibility to infection would elucidate why mycoplankton abundance increases during the polar night. Further, future studies confirming the potential predatory interactions would allow us to confirm the validity of the food web model. For example, do we observe decreases in zoospore size during the polar night and do predatory protists show preference towards this prey? Lastly, it may be prudent to include the V7-V8 primer set in future studies of the McMurdo Dry Valleys as these capture a greater diversity of heterotrophic protists and fungi that are otherwise missed with the commonly used V9 primer set.

5) References

Agha, Ramsy, Alina Gross, Thomas Rohrlack, and Justyna Wolinska. 2018. "Adaptation of a Chytrid Parasite to Its Cyanobacterial Host Is Hampered by Host Intraspecific Diversity." *Frontiers in Microbiology* 9 (May). https://doi.org/10.3389/fmicb.2018.00921.

Amaral-Zettler, Linda A., Elizabeth A. McCliment, Hugh W. Ducklow, and Susan M. Huse. 2009. "A Method for Studying Protistan Diversity Using Massively Parallel Sequencing of V9 Hypervariable Regions of Small-Subunit Ribosomal RNA Genes." *PloS One* 4 (7): e6372. https://doi.org/10.1371/journal.pone.0006372.

Bellingeri, M., D. Bevacqua, F. Scotognella, R. Alfieri, and D. Cassi. 2020. "A Comparative Analysis of Link Removal Strategies in Real Complex Weighted Networks." *Scientific Reports* 10 (1): 3911. https://doi.org/10.1038/s41598-020-60298-7.

Boyse, Elizabeth, Kevin P. Robinson, Ian M. Carr, Elena Valsecchi, Maria Beger, and Simon J. Goodman. 2025. "Inferring Species Interactions From Co-occurrence Networks With Environmental DNA Metabarcoding Data in a Coastal Marine Food Web." *Molecular Ecology* 34 (7): e17701. https://doi.org/10.1111/mec.17701.

Busch, Anna, and Sebastian and Hess. 2022. "Sunscreen Mucilage: A Photoprotective Adaptation Found in Terrestrial Green Algae (Zygnematophyceae)." *European Journal of Phycology* 57 (1): 107–24. https://doi.org/10.1080/09670262.2021.1898677.

Callahan, Benjamin J., Paul J. McMurdie, Michael J. Rosen, Andrew W. Han, Amy Jo A. Johnson, and Susan P. Holmes. 2016. "DADA2: High-Resolution Sample Inference from Illumina Amplicon Data." *Nature Methods* 13 (7): 581–83. https://doi.org/10.1038/nmeth.3869.

Cameron, Ellen S., Philip J. Schmidt, Benjamin J.-M. Tremblay, Monica B. Emelko, and Kirsten M. Müller. 2021. "Enhancing Diversity Analysis by Repeatedly Rarefying next Generation Sequencing Data Describing Microbial Communities." *Scientific Reports* 11 (1): 22302. https://doi.org/10.1038/s41598-021-01636-1.

Chen, Ming, Honghui Gao, and Jimin Zhang. 2024. "Mycoloop: Modeling Phytoplankton–Chytrid–Zooplankton Interactions in Aquatic Food Webs." *Mathematical Biosciences* 368 (February):109134. https://doi.org/10.1016/j.mbs.2023.109134.

Chen, Shilin, Hui Yao, Jianping Han, Chang Liu, Jingyuan Song, Linchun Shi, Yingjie Zhu, et al. 2010. "Validation of the ITS2 Region as a Novel DNA Barcode for Identifying Medicinal Plant Species." *PLOS ONE* 5 (1): e8613. https://doi.org/10.1371/journal.pone.0008613.

Cornwallis, Charlie K., Maria Svensson-Coelho, Markus Lindh, Qinyang Li, Franca Stábile, Lars-Anders Hansson, and Karin Rengefors. 2023. "Single-Cell Adaptations Shape Evolutionary Transitions to Multicellularity in Green Algae." *Nature Ecology & Evolution* 7 (6): 889–902. https://doi.org/10.1038/s41559-023-02044-6.

Corsaro, Daniele, Julia Walochnik, Danielle Venditti, Jörg Steinmann, Karl-Dieter Müller, and Rolf Michel. 2014. "Microsporidia-like Parasites of Amoebae Belong to the Early Fungal Lineage Rozellomycota." *Parasitology Research* 113 (5): 1909–18. https://doi.org/10.1007/s00436-014-3838-4.

Dumack, Kenneth, Julia Pundt, and Michael Bonkowski. 2019. "Food Choice Experiments Indicate Selective Fungivorous Predation in Fisculla Terrestris (Thecofilosea, Cercozoa)." *Journal of Eukaryotic Microbiology* 66 (3): 525–27. https://doi.org/10.1111/jeu.12680.

Estrada, Ernesto. 2007. "Characterization of Topological Keystone Species: Local, Global and 'Meso-Scale' Centralities in Food Webs." *Ecological Complexity* 4 (1): 48–57. https://doi.org/10.1016/j.ecocom.2007.02.018.

Fountain, Andrew G., Joseph S. Levy, Michael N. Gooseff, and David Van Horn. 2014. "The McMurdo Dry Valleys: A Landscape on the Threshold of Change." *Geomorphology*, Permafrost and periglacial research in Antarctica: New results and perspectives, 225 (November):25–35. https://doi.org/10.1016/j.geomorph.2014.03.044.

Freilich, Mara A., Evie Wieters, Bernardo R. Broitman, Pablo A. Marquet, and Sergio A. Navarrete. 2018. "Species Co-Occurrence Networks: Can They Reveal Trophic and Non-Trophic Interactions in Ecological Communities?" *Ecology* 99 (3): 690–99. https://doi.org/10.1002/ecy.2142.

Friedman, Jonathan, and Eric J. Alm. 2012. "Inferring Correlation Networks from Genomic Survey Data." *PLOS Computational Biology* 8 (9): e1002687. https://doi.org/10.1371/journal.pcbi.1002687.

Gałązka, Anna, Anna Marzec-Grządziel, Jarosław Grządziel, Milan Varsadiya, and Łukasz Pawlik. 2022. "Fungal Genetic Biodiversity and Metabolic Activity as an Indicator of Potential Biological Weathering and Soil Formation – Case Study of towards a Better Understanding of Earth System Dynamics." *Ecological Indicators* 141 (August):109136. https://doi.org/10.1016/j.ecolind.2022.109136.

Gleason, Frank H., Laura T. Carney, Osu Lilje, and Sally L. Glockling. 2012. "Ecological Potentials of Species of *Rozella* (Cryptomycota)." *Fungal Ecology* 5 (6): 651–56. https://doi.org/10.1016/j.funeco.2012.05.003.

Gleason, Frank H., Maiko Kagami, Emilie Lefevre, and Telesphore Sime-Ngando. 2008. "The Ecology of Chytrids in Aquatic Ecosystems: Roles in Food Web Dynamics." *Fungal Biology Reviews* 22 (1): 17–25. https://doi.org/10.1016/j.fbr.2008.02.001.

Gooseff, Michael N., John E. Barrett, Byron J. Adams, Peter T. Doran, Andrew G. Fountain, W. Berry Lyons, Diane M. McKnight, et al. 2017. "Decadal Ecosystem Response to an Anomalous Melt Season in a Polar Desert in Antarctica." *Nature Ecology & Evolution* 1 (9): 1334–38. https://doi.org/10.1038/s41559-017-0253-0.

Green, William J., and W. Berry Lyons. 2009. "The Saline Lakes of the McMurdo Dry Valleys, Antarctica." *Aquatic Geochemistry* 15 (1): 321–48. https://doi.org/10.1007/s10498-008-9052-1.

Grossart, Hans-Peter, Silke Van den Wyngaert, Maiko Kagami, Christian Wurzbacher, Michael Cunliffe, and Keilor Rojas-Jimenez. 2019. "Fungi in Aquatic Ecosystems." *Nature Reviews Microbiology* 17 (6): 339–54. https://doi.org/10.1038/s41579-019-0175-8.

Grossart, Hans-Peter, Christian Wurzbacher, Timothy Y. James, and Maiko Kagami. 2016. "Discovery of Dark Matter Fungi in Aquatic Ecosystems Demands a Reappraisal of the Phylogeny and Ecology of Zoosporic Fungi." *Fungal Ecology*, Aquatic Fungi, 19 (February):28–38. https://doi.org/10.1016/j.funeco.2015.06.004.

Grossmann, Lars, Christina Bock, Michael Schweikert, and Jens Boenigk. 2016. "Small but Manifold – Hidden Diversity in 'Spumella-like Flagellates.'" *Journal of Eukaryotic Microbiology* 63 (4): 419–39. https://doi.org/10.1111/jeu.12287.

Guillou, Laure, Dipankar Bachar, Stéphane Audic, David Bass, Cédric Berney, Lucie Bittner, Christophe Boutte, et al. 2013. "The Protist Ribosomal Reference Database (PR2): A Catalog of Unicellular Eukaryote Small Sub-Unit rRNA Sequences with Curated Taxonomy." *Nucleic Acids Research* 41 (D1): D597–604. https://doi.org/10.1093/nar/gks1160.

Guimerà, Roger, and Luís A. Nunes Amaral. 2005. "Functional Cartography of Complex Metabolic Networks." *Nature* 433 (7028): 895–900. https://doi.org/10.1038/nature03288.

Herron, Matthew D., Joshua M. Borin, Jacob C. Boswell, Jillian Walker, I.-Chen Kimberly Chen, Charles A. Knox, Margrethe Boyd, Frank Rosenzweig, and William C. Ratcliff. 2019. "De Novo Origins of Multicellularity in Response to Predation." *Scientific Reports* 9 (1): 2328. https://doi.org/10.1038/s41598-019-39558-8.

Ilicic, Doris, and Hans-Peter Grossart. 2022. "Basal Parasitic Fungi in Marine Food Webs—A Mystery Yet to Unravel." *Journal of Fungi* 8 (2): 114. https://doi.org/10.3390/jof8020114.

IMIS, VLIZ-. n.d. "WoRMS - World Register of Marine Species - IMIS." Accessed April 2, 2025. https://www.marinespecies.org/imis.php?dasid=1447&doiid=170.

Irion, Solène, Urania Christaki, Hugo Berthelot, Stéphane L'Helguen, and Ludwig Jardillier. 2021. "Small Phytoplankton Contribute Greatly to CO2-Fixation after the Diatom Bloom in the Southern Ocean." *The ISME Journal* 15 (9): 2509–22. https://doi.org/10.1038/s41396-021-00915-z.

Jiao, N., Y. Zhang, K. Zhou, Q. Li, M. Dai, J. Liu, J. Guo, and B. Huang. 2014. “Revisiting the CO_2 ‘Source’ Problem in Upwelling Areas – a Comparative Study on Eddy Upwellings in the South China Sea.” *Biogeosciences* 11 (9): 2465–75. https://doi.org/10.5194/bg-11-2465-2014.

Jiao, Nianzhi, Tingwei Luo, Quanrui Chen, Zhao Zhao, Xilin Xiao, Jihua Liu, Zhimin Jian, et al. 2024. “The Microbial Carbon Pump and Climate Change.” *Nature Reviews Microbiology* 22 (7): 408–19. https://doi.org/10.1038/s41579-024-01018-0.

Johnsen, Geir, Eva Leu, and Rolf Gradinger. 2020. “Marine Micro- and Macroalgae in the Polar Night.” In *POLAR NIGHT Marine Ecology: Life and Light in the Dead of Night*, edited by Jørgen Berge, Geir Johnsen, and Jonathan H. Cohen, 67–112. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-030-33208-2_4.

Johnson, Kenneth S., and Mariana B. Bif. 2021. “Constraint on Net Primary Productivity of the Global Ocean by Argo Oxygen Measurements.” *Nature Geoscience* 14 (10): 769–74. https://doi.org/10.1038/s41561-021-00807-z.

Kaczmarek, Agata, and Mieczysława I. Boguś. 2021. “Fungi of Entomopathogenic Potential in Chytridiomycota and Blastocladiomycota, and in Fungal Allies of the Oomycota and Microsporidia.” *IMA Fungus* 12 (1): 29. https://doi.org/10.1186/s43008-021-00074-y.

Kagami, Maiko, Takeshi Miki, and Gaku Takimoto. 2014. “Mycoloop: Chytrids in Aquatic Food Webs.” *Frontiers in Microbiology* 5 (April). https://doi.org/10.3389/fmicb.2014.00166.

Khanipour Roshan, Samira, Kenneth Dumack, Michael Bonkowski, Peter Leinweber, Ulf Karsten, and Karin Glaser. 2021. “Taxonomic and Functional Diversity of Heterotrophic Protists (Cercozoa and Endomyxa) from Biological Soil Crusts.” *Microorganisms* 9 (2): 205. https://doi.org/10.3390/microorganisms9020205.

Klawonn, Isabell, Silke Van den Wyngaert, Alma E. Parada, Nestor Arandia-Gorostidi, Martin J. Whitehouse, Hans-Peter Grossart, and Anne E. Dekas. 2021. “Characterizing the ‘Fungal Shunt’: Parasitic Fungi on Diatoms Affect Carbon Flow and Bacterial Communities in Aquatic Microbial Food Webs.” *Proceedings of the National Academy of Sciences* 118 (23): e2102225118. https://doi.org/10.1073/pnas.2102225118.

Lee, Hanbyul, Kyuin Hwang, Ahnna Cho, Soyeon Kim, Minkyung Kim, Rachael Morgan-Kiss, John C. Priscu, Kyung Mo Kim, and Ok-Sun Kim. 2024. “Microbial Assemblages and Associated Biogeochemical Processes in Lake Bonney, a Permanently Ice-Covered Lake in the McMurdo Dry Valleys, Antarctica.” *Environmental Microbiome* 19 (1): 60. https://doi.org/10.1186/s40793-024-00605-1.

Lee, Kiseok Keith, Hyun Kim, and Yong-Hwan Lee. 2022. “Cross-Kingdom Co-Occurrence Networks in the Plant Microbiome: Importance and Ecological Interpretations.” *Frontiers in Microbiology* 13 (July):953300. https://doi.org/10.3389/fmicb.2022.953300.

Letcher, Peter M., and Martha J. Powell. 2018. “A Taxonomic Summary and Revision of Rozella (Cryptomycota).” *IMA Fungus* 9 (July):383–99. https://doi.org/10.5598/imafungus.2018.09.02.09.

———. 2019. “A Taxonomic Summary of Aphelidiaceae.” *IMA Fungus* 10 (1): 4. https://doi.org/10.1186/s43008-019-0005-7.

Liu, Chi, Yaoming Cui, Xiangzhen Li, and Minjie Yao. 2021. “Microeco: An R Package for Data Mining in Microbial Community Ecology.” *FEMS Microbiology Ecology* 97 (2): fiaa255. https://doi.org/10.1093/femsec/fiaa255.

Liu, Chi, Chaonan Li, Yanqiong Jiang, Raymond J. Zeng, Minjie Yao, and Xiangzhen Li. 2023. “A Guide for Comparing Microbial Co-Occurrence Networks.” *iMeta* 2 (1): e71. https://doi.org/10.1002/imt2.71.

Marchetta, Alessia, Maria Papale, Alessandro Ciro Rappazzo, Carmen Rizzo, Antonio Camacho, Carlos Rochera, Maurizio Azzaro, Clara Urzì, Angelina Lo Giudice, and Filomena De Leo. 2023. “A Deep Insight into the Diversity of Microfungal Communities in Arctic and Antarctic Lakes.” *Journal of Fungi* 9 (11): 1095. https://doi.org/10.3390/jof9111095.

Martínez Rendón, Cristina, Christina Braun, Maria Kappelsberger, Jens Boy, Angélica Casanova-Katny, Karin Glaser, and Kenneth Dumack. 2025. “Enhancing Microbial Predator–Prey Detection with Network and Trait-Based Analyses.” *Microbiome* 13 (1): 37. https://doi.org/10.1186/s40168-025-02035-8.

McMurdie, Paul J., and Susan Holmes. 2013. “Phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data.” *PLOS ONE* 8 (4): e61217. https://doi.org/10.1371/journal.pone.0061217.

———. 2014. “Waste Not, Want Not: Why Rarefying Microbiome Data Is Inadmissible.” *PLOS Computational Biology* 10 (4): e1003531. https://doi.org/10.1371/journal.pcbi.1003531.

Mitchell, Kendra R, and Cristina D Takacs-Vesbach. 2008. “A Comparison of Methods for Total Community DNA Preservation and Extraction from Various Thermal Environments.” *Journal of Industrial Microbiology and Biotechnology* 35 (10): 1139–47. https://doi.org/10.1007/s10295-008-0393-y.

Mitra, Aditee, David A. Caron, Emile Faure, Kevin J. Flynn, Suzana Gonçalves Leles, Per J. Hansen, George B. McManus, et al. 2023. “The Mixoplankton Database (MDB): Diversity of Photo-phago-trophic Plankton in Form, Function, and Distribution across the Global Ocean.” *Journal of Eukaryotic Microbiology* 70 (4): e12972. https://doi.org/10.1111/jeu.12972.

Morgan-Kiss, R. M., M. P. Lizotte, W. Kong, and J. C. Priscu. 2016. “Photoadaptation to the Polar Night by Phytoplankton in a Permanently Ice-Covered Antarctic Lake.” *Limnology and Oceanography* 61 (1): 3–13. https://doi.org/10.1002/lno.10107.

Morgan-Kiss, Rachael M., Devon Popson, Rochelle Pereira, Jenna Dolhi-Binder, Amber Teufel, Wei Li, Isha Kalra, Shasten Sherwell, Emily Reynebeau, and Cristina Takacs-Vesbach. 2024. “Sentinel Protist Taxa of the McMurdo Dry Valley Lakes, Antarctica: A Review.” *Frontiers in Ecology and Evolution* 12 (March). https://doi.org/10.3389/fevo.2024.1323472.

Naranjo-Ortiz, Miguel A., and Toni Gabaldón. 2019. “Fungal Evolution: Diversity, Taxonomy and Phylogeny of the Fungi.” *Biological Reviews* 94 (6): 2101–37. https://doi.org/10.1111/brv.12550.

Nguyen, Nhu H., Zewei Song, Scott T. Bates, Sara Branco, Leho Tedersoo, Jon Menke, Jonathan S. Schilling, and Peter G. Kennedy. 2016. “FUNGuild: An Open Annotation Tool for Parsing Fungal Community Datasets by Ecological Guild.” *Fungal Ecology* 20 (April):241–48. https://doi.org/10.1016/j.funeco.2015.06.006.

Obryk, M. K., P. T. Doran, A. G. Fountain, M. Myers, and C. P. McKay. 2020. “Climate From the McMurdo Dry Valleys, Antarctica, 1986–2017: Surface Air Temperature Trends and Redefined Summer Season.” *Journal of Geophysical Research: Atmospheres* 125 (13): e2019JD032180. https://doi.org/10.1029/2019JD032180.

Oña, Leonardo, Shryli K. Shreekar, and Christian Kost. 2025. “Disentangling Microbial Interaction Networks.” *Trends in Microbiology* 0 (0). https://doi.org/10.1016/j.tim.2025.01.013.

Palmer, J. R., and I. J. Totterdell. 2001. “Production and Export in a Global Ocean Ecosystem Model.” *Deep Sea Research Part I: Oceanographic Research Papers* 48 (5): 1169–98. https://doi.org/10.1016/S0967-0637(00)00080-7.

Parr, Cynthia S., Nathan Wilson, Patrick Leary, Katja S. Schulz, Kristen Lans, Lisa Walley, Jennifer A. Hammock, et al. 2014. “The Encyclopedia of Life v2: Providing Global Access to Knowledge About Life on Earth.” *Biodiversity Data Journal*, no. 2 (April), e1079. https://doi.org/10.3897/BDJ.2.e1079.

Patriarche, J. D., J. C. Priscu, C. Takacs-Vesbach, L. Winslow, K. F. Myers, H. Buelow, R. M. Morgan-Kiss, and P. T. Doran. 2021. “Year-Round and Long-Term Phytoplankton Dynamics in Lake Bonney, a Permanently Ice-Covered Antarctic Lake.” *Journal of Geophysical Research: Biogeosciences* 126 (4): e2020JG005925. https://doi.org/10.1029/2020JG005925.

Penderson, Thomas. 2024. “An Implementation of Grammar of Graphics for Graphs and Networks.” 2024. https://github.com/thomasp85/ggraph, https://ggraph.data-imaginist.com.

Rasconi, Serena, Nathalie Niquil, and Télesphore Sime-Ngando. 2012. “Phytoplankton Chytridiomycosis: Community Structure and Infectivity of Fungal Parasites in Aquatic Ecosystems.” *Environmental Microbiology* 14 (8): 2151–70. https://doi.org/10.1111/j.1462-2920.2011.02690.x.

Richter, Daniel J., and Frank Nitsche. 2017. “Choanoflagellatea.” In *Handbook of the Protists*, edited by John M. Archibald, Alastair G.B. Simpson, and Claudio H. Slamovits, 1479–96. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-28149-0_5.

Rojas-Jimenez, Keilor, Christian Wurzbacher, Elizabeth Charlotte Bourne, Amy Chiuchiolo, John C. Priscu, and Hans-Peter Grossart. 2017. “Early Diverging Lineages within Cryptomycota and Chytridiomycota Dominate the Fungal Communities in Ice-Covered Lakes of the McMurdo Dry Valleys, Antarctica.” *Scientific Reports* 7 (November):15348. https://doi.org/10.1038/s41598-017-15598-w.

Sampaio, José Paulo, Robert Bauer, Dominik Begerow, and Franz Oberwinkler. 1999. “Occultifur Externus Sp. Nov., a New Species of Simple-Pored Auricularioid Heterobasidiomycete from Plant Litter in Portugal.” *Mycologia* 91 (6): 1094–1101. https://doi.org/10.2307/3761639.

Schiwitza, Sabine, Hartmut Arndt, and Frank Nitsche. 2019. “First Description of an Euryoecious Acanthoecid Choanoflagellate Species, *Enibas Tolerabilis* Gen. et Sp. Nov. from a Salar in the Chilean Andes Based on Morphological and Transcriptomic Data.” *European Journal of Protistology* 67 (February):106–13. https://doi.org/10.1016/j.ejop.2018.11.004.

Scholz, Bettina, Frithjof C. Küpper, Wim Vyverman, Halldór G. Ólafsson, and Ulf Karsten. 2017. “Chytridiomycosis of Marine Diatoms—The Role of Stress Physiology and Resistance in Parasite-Host Recognition and Accumulation of Defense Molecules.” *Marine Drugs* 15 (2): 26. https://doi.org/10.3390/md15020026.

Silsbe, Greg M., Michael J. Behrenfeld, Kimberly H. Halsey, Allen J. Milligan, and Toby K. Westberry. 2016. “The CAFE Model: A Net Production Model for Global Ocean Phytoplankton.” *Global Biogeochemical Cycles* 30 (12): 1756–77. https://doi.org/10.1002/2016GB005521.

Simberloff, Daniel, and Tamar Dayan. 1991. “The Guild Concept and the Structure of Ecological Communities.” *Annual Review of Ecology and Systematics* 22:115–43.

Sime - Ngando, Télesphore. 2012. “Phytoplankton Chytridiomycosis: Fungal Parasites of Phytoplankton and Their Imprints on the Food Web Dynamics.” *Frontiers in Microbiology* 3 (October). https://doi.org/10.3389/fmicb.2012.00361.

Stoeck, Thorsten, David Bass, Markus Nebel, Richard Christen, Meredith D. M. Jones, Hans-Werner Breiner, and Thomas A. Richards. 2010. “Multiple Marker Parallel Tag Environmental DNA Sequencing Reveals a Highly Complex Eukaryotic Community in Marine Anoxic Water.” *Molecular Ecology* 19 Suppl 1 (March):21–31. https://doi.org/10.1111/j.1365-294X.2009.04480.x.

Tedersoo, Leho, Sten Anslan, Mohammad Bahram, Sergei Põlme, Taavi Riit, Ingrid Liiv, Urmas Kõljalg, et al. 2015. “Shotgun Metagenomes and Multiple Primer Pair-Barcode Combinations of Amplicons Reveal Biases in Metabarcoding Analyses of Fungi.” *MycoKeys* 10 (May):1–43. https://doi.org/10.3897/mycokeys.10.4852.

Thongthaisong, Patch, Minoru Kasada, Hans-Peter Grossart, and Sabine Wollrab. 2022. “Critical Role of Parasite-mediated Energy Pathway on Community Response to Nutrient Enrichment.” *Ecology and Evolution* 12 (12): e9622. https://doi.org/10.1002/ece3.9622.

Tranvik, Lars J., Jonathan J. Cole, and Yves T. Prairie. 2018. “The Study of Carbon in Inland Waters—from Isolated Ecosystems to Players in the Global Carbon Cycle.” *Limnology and Oceanography Letters* 3 (3): 41–48. https://doi.org/10.1002/lol2.10068.

Van den Wyngaert, Silke, Lars Ganzert, Kensuke Seto, Keilor Rojas-Jimenez, Ramsy Agha, Stella A. Berger, Jason Woodhouse, et al. 2022. “Seasonality of Parasitic and Saprotrophic Zoosporic Fungi: Linking Sequence Data to Ecological Traits.” *The ISME Journal* 16 (9): 2242–54. https://doi.org/10.1038/s41396-022-01267-y.

Van Den Wyngaert, Silke, Martin J Kainz, and Robert Ptacnik. 2022. “Mucilage Protects the Planktonic Desmid Staurodesmus Sp. against Parasite Attack by a Chytrid Fungus.” *Journal of Plankton Research* 45 (1): 3–14. https://doi.org/10.1093/plankt/fbac071.

Vick-Majors, Trista J., John C. Priscu, and Linda A Amaral-Zettler. 2014. “Modular Community Structure Suggests Metabolic Plasticity during the Transition to Polar Night in Ice-Covered Antarctic Lakes.” *The ISME Journal* 8 (4): 778–89. https://doi.org/10.1038/ismej.2013.190.

Wietz, Matthias, Christina Bienhold, Katja Metfies, Sinhué Torres-Valdés, Wilken-Jon von Appen, Ian Salter, and Antje Boetius. 2021. “The Polar Night Shift: Seasonal Dynamics and Drivers of Arctic Ocean Microbiomes Revealed by Autonomous Sampling.” *ISME Communications* 1 (1): 1–12. https://doi.org/10.1038/s43705-021-00074-4.

Winslow, Luke, Hilary Dugan, Heather Buelow, Kyle Cronin, John Priscu, Cristina Takacs-Vesbach, and Peter Doran. 2014. “Autonomous Year-Round Sampling and Sensing to Explore the Physical and Biological Habitability of Permanently Ice-Covered Antarctic Lakes.” *Marine Technology Society Journal* 48 (October). https://doi.org/10.4031/MTSJ.48.5.6.

Wurzbacher, Christian, Ellen Larsson, Johan Bengtsson-Palme, Silke Van den Wyngaert, Sten Svantesson, Erik Kristiansson, Maiko Kagami, and R. Henrik Nilsson. 2019. “Introducing Ribosomal Tandem Repeat Barcoding for Fungi.” *Molecular Ecology Resources* 19 (1): 118–27. https://doi.org/10.1111/1755-0998.12944.

Yuan, Mengting Maggie, Xue Guo, Linwei Wu, Ya Zhang, Naijia Xiao, Daliang Ning, Zhou Shi, et al. 2021. “Climate Warming Enhances Microbial Network Complexity and Stability.” *Nature Climate Change* 11 (4): 343–48. https://doi.org/10.1038/s41558-021-00989-9.