Department of Integrated Biosciences
Graduate School of Frontier Sciences
The University of Tokyo

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Master's Thesis

Examination of marine fungal community in suspended and sinking particle fractions using 18S rRNA gene amplicon sequencing during a spring phytoplankton bloom in sub-Arctic water layers

（亜寒帯春季ブルーム期における懸濁・沈降性海洋菌類群集の 18S rRNA 遺伝子アンプリコンシーケンス解析）

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Abstract

Introduction

Fungi have been known to play important roles in nutrient and element recycling in terrestrial ecosystems, but their role in food webs and biogeochemical cycles in marine environments remains poorly understood. In marine environments, sinking particles drive the biological carbon pump which refers to the biological processes that transform atmospheric CO_2 into various forms of carbon and eventually sequesters large quantities of carbon in the deep ocean. Protozoans and bacteria are known to colonize sinking particles and affect their decomposition and transformation; however, data on the fungal community associated with sinking particles are scarce. The aim of this study was to characterize fungal communities in suspended and sinking particle fractions during a spring phytoplankton bloom in a subarctic region. I collected samples during two cruises conducted in March and May using a sampling device named Marine Snow Catcher (OSIL Ltd, U.K.). Particle samples were collected at three different layers, including the chlorophyll maximum layer (CM), pycnocline (PC), and bottom boundary layer (BBL). Fungal communities were examined using the 18S rRNA gene amplicon sequencing method.

Results and Discussion

Fungi were detected in most (88%) of the samples. The phylum *Chytridiomycota* was the most abundant in terms of ASV number (Fig. 1) and more than half of the prevalent ASVs were affiliated with *Chytridiomycota*. These results differ from previous observations suggesting that the marine fungal community is generally dominated by the subkingdom *Dikarya* (*Ascomycota* and *Basidiomycota*). However, they support an emerging notion that the basal fungal phyla such as *Chytridiomycota* may play an important role in some marine ecosystems. Nonmetric multidimensional scaling (NMDS) ordination and permutational analysis of variance (PERMANOVA) revealed that fungal communities differed significantly ($P = 0.001$) between March and May (Fig. 2), which represent different bloom succession stages. Fungal community composition also differed significantly ($P = 0.007$) between the layers (Fig. 3). Notably, alpha diversity for fungal communities, as evaluated by Shannon and Simpson diversity indices, was higher in the BBL than the CM, suggesting that bottom sediment resuspension enhanced fungal diversity in the BBL. The difference of fungal communities between sinking and suspended fractions was insignificant ($P = 0.19$). Analyses of the distribution pattern of the prevalent ASVs revealed some notable features. Some ASVs were generalists which occurred regardless of month, layer and fraction, whereas other ASVs were specialists which were associated with specific months and layers. Spearman correlation analysis identified a covariation of three specialist ASVs affiliated with *Chytridiomycota (Rhizophydiales),* suggesting that they had similar niche-preference.

Conclusion

My results showed that fungi are ubiquitous in the studied region. The high abundance of *Chytridiomycota* suggests the need to further investigate basal fungal phyla. The occurrence of fungi in the sinking fractions suggest that fungi potentially play a role in the regulation of the biological carbon pump. Future studies are required to clarify the metabolic activity and ecological function of fungal communities.

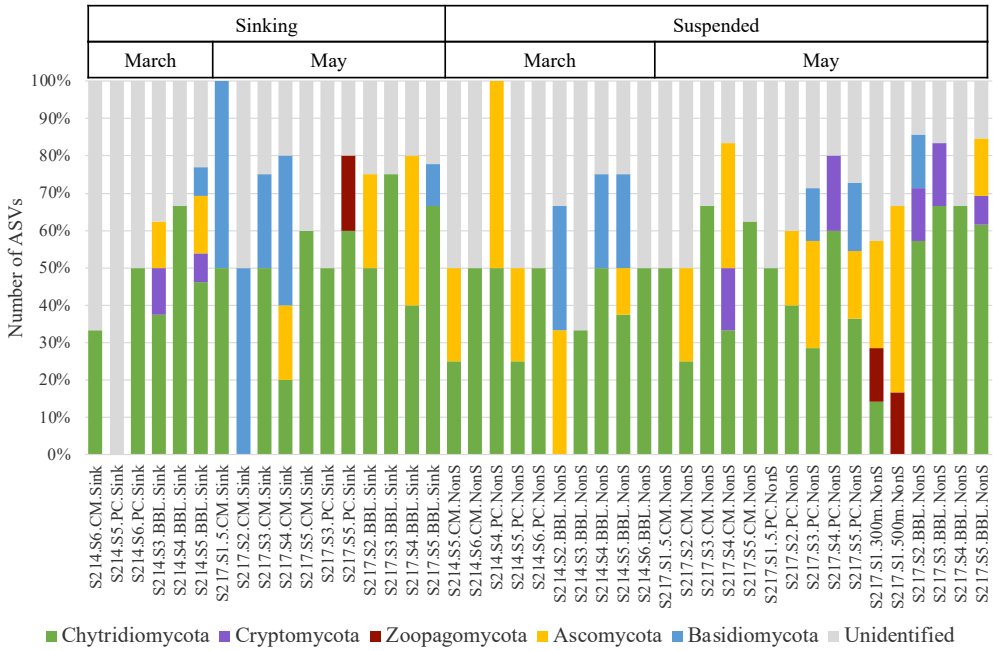

Fig. 1: Taxonomic composition at the phylum level at each station in terms of ASV numbers. Percentage of each phylum is as follows: 38% for *Chytridiomycota*, 14% to *Ascomycota*, 14% to *Basidiomycota*, 7% to *Cryptomycota*, 3% to *Zoopagomycota* and 24% to unidentified fungi.

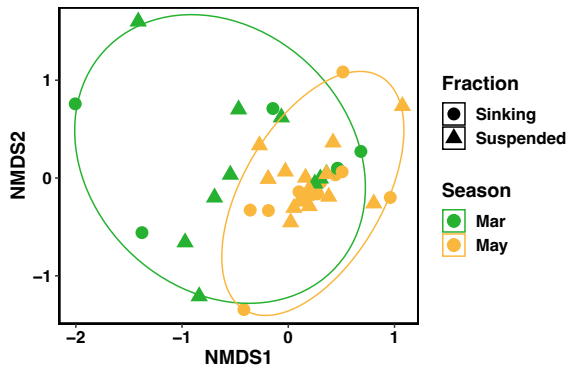

Fig. 2: NMDS ordination plot colour-coded for months (stress value = 0.1). PERMANOVA analysis showed that fungal communities differed by month (P = 0.001)

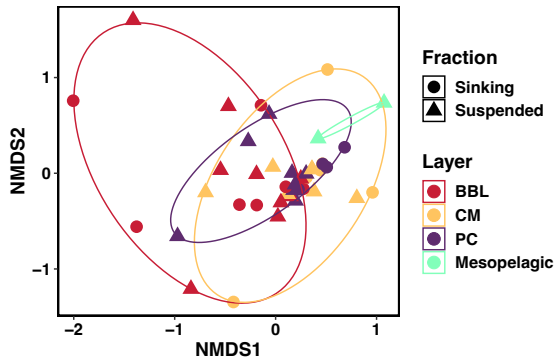

Fig. 3: NMDS ordination plot colour-coded for layers (stress value = 0.1). PERMANOVA analysis showed that fungal communities differed by layer (P = 0.007)

Introduction

Fungi have been known to play important roles in nutrient and element recycling in terrestrial ecosystems (Ehrlich, 2006). However, fungi in marine environments have been understudied and their role in food webs and biogeochemical cycles remains poorly understood (Amend et al., 2019; Ilicic and Grossart, 2022). This paucity of knowledge on marine fungi is partly due to difficulties and limitations with traditional methods using microscopy and cultivation (Amend et al., 2019). Detecting, isolating and culturing marine fungi have been a challenging task, being hampered by the complex life stages and environmental requirements of marine fungi (Gonçalves et al., 2022). During the past decade, with the advent of next generation sequencing, studies have begun to reveal that marine fungi are distributed in diverse marine environments (Amend et al., 2019). Investigators have also found the presence of the so-called "Dark Matter Fungi" which are only known by their sequence data (Grossart et al. 2016; Ilicic and Grossart, 2022). However, data and studies on marine fungi are still limited: with less than 2,000 marine fungal species currently described (https://www.marinefungi.org, accessed on 17 December 2022) out of the estimated 10,000 to 12,500 species of marine fungi (Jones, 2011).

Unlike animal and plant sequencing, there is no universally accepted DNA barcode, or target sequence region for fungi (Schoch et al., 2012). Sequencing studies for fungi usually target the 18S rRNA gene or the internal transcribed spacer (ITS) region, the latter being the proposed universal barcode for fungi (Schoch et al., 2012). However, ITS-sequencing is not ideal for fungi in marine environments as it readily coamplifies with other eukaryotes such as zooplankton and invertebrates, which are generally more abundant than marine fungi, potentially masking the presence of the relatively rare fungi (Banos et al., 2018). Furthermore, ITS primers and databases tend to be biased towards terrestrial lineages of fungi such as the *Dikarya* and consequentially have

limited power in identifying other fungal groups, particularly the basal lineages such as *Chytridiomycota* (Banos et al., 2018). 18S rRNA genes are more conserved and therefore, suitable for phylogenetic analysis at higher taxonomic levels (e.g. phylum), but due to the low variability, classification at the lower taxonomic level (e.g. genus and species) is compromised (Frau et al., 2019). Two recent studies (Banos et al., 2020; Priest et al., 2021) have reported success using the 18S rRNA primer FF390/FR1 (Vainio and Hantula, 2000) with blocking oligonucleotides (Banos et al., 2018) for marine fungi sequencing surveys.

In marine environments, sequencing studies have found that *Ascomycota* and *Basidiomycota*, which make up the subkingdom *Dikarya*, often dominate fungal communities (Morales et al., 2019; Picard, 2017; Sen et al., 2021; Tisthammer et al., 2016). For example, in the global marine fungi dataset (International Census of Marine Microbes Projects) for sediment and the water column (Amaral-Zettler et al., 2010), which identified 2200 operational taxonomic units (OTUs), *Dikarya* were found to be the most abundant phyla, whereas other phyla belonging to *Chytridiomycota* and *Cryptomycota* were notably sparse (Tisthammer et al., 2016). Sen et al. (2021) sequenced three target regions, 18S, ITS1 and ITS2 in Shenzhen Bay, Daya Bay and the South China Sea. They found that regardless of the studied region, *Dikarya* were dominant, accounting for 94% for 18S, 88% for ITS1 and 98% for ITS2 of the total fungal abundance. Time-series studies have shown that fungal communities are dynamic and taxonomic abundance changes over time (Banos et al., 2020; Priest et al., 2021; Tisthammer et al., 2016). In the German Bight, sequencing over a year showed that *Dikarya* (*Ascomycota*) were dominant in the majority of the samples, whereas *Chytridiomycota* were dominant in only a few samples (Banos et al., 2020). However, other studies have found that *Chytridiomycota* can be abundant in some marine environments. (Comeau et al., 2016; Jeffries et al., 2016; Richards et al., 2015). Notably, Comeau

et al. (2016) found that two-thirds of fungal sequences belonged to *Chytridiomycota* in Arctic seawaters (Comeau et al., 2016). *Chytridiomycota* are "early diverging lineages" of fungi and their diversity, distribution and ecology in marine environments are still poorly understood (Amend et al. 2019).

Fungi have multiple trophic behaviours and ecological roles. Broadly, they can be divided into saprotrophs (eating dead organic matter) and biotrophs (eating living cells), where biotrophic behaviour includes parasitism (Ilicic and Grossart, 2022). Infection by marine fungi has been reported for many lineages including mammals, invertebrates, corals, sponges, macroalgae and microalgae (Higgins, 2000; Ilicic and Grossart, 2022; Richards et al., 2012). With regards to marine phytoplankton, studies to date have only documented infections by members of *Chytridiomycota* (Garvetto et al., 2019; Gutiérrez et al., 2016; Lepère et al., 2016). These studies have generally focused on *Chytridiomycota* infection of diatoms, however, they may also infect dinoflagellates (Lepelletier et al., 2014). Fungal parasitism has been studied using *Chytridiomycota* as a model system (Ilicic and Grossart, 2022). In brief, *Chytridiomycota* in the zoospore stage search for, then attach to a host using rhizoids to anchor themselves and siphon nutrients from the host to grow and expand into a sporangium which contains zoospores that will be released for the next generation (Fig. 1). Parasitism is especially important during the phytoplankton bloom. Parasitic fungi are crucial in regulating phytoplankton populations (Frenken et al., 2017). In freshwater environments, such top-down control by fungi via infection and parasitism of phytoplankton can lead to retardation or even suppression of bloom events (Frenken et al., 2017). Phytoplankton species composition shifts due to the selective effects of fungal infection, leading to successional changes in the community (Frenken et al., 2017). Through parasitism, fungi affect the food chain by acting as a trophic link. In a process known as the

mycoloop, parasitic fungi infect unpalatable phytoplankton and fragmentize them so that they are consumable by zooplankton (Kagami et al., 2014). This has been well characterized in freshwater environments but is yet to be proven in marine environments.

As saprophytes, fungi on land are crucial in degradation of recalcitrant organic material like cellulose and lignin, thus it has been speculated that marine fungi may also play a similar role (Sun et al., 2017). *Malassezia* (*Basidiomycota*) and *Cladosporium* (*Ascomycota*) were shown to be active saprotrophs in coastal environments (Cunliffe et al., 2017). Due to their osmotrophic feeding mode, fungi rely on an extensive toolkit of enzymes to degrade organic matter. Enzymes including chitinase, cellulase complex, ligninase, and laminarinase have been found in marine fungi (Clipson et al., 2005). Considering the saprotrophic abilities of terrestrial fungi, it is plausible that they may contribute to organic matter mineralization and transformation in marine environments as well (Ortega-Arbulú et al., 2019). Currently, the contribution of fungal saprotrophs to marine biogeochemical cycles remains unclear. As heterotrophs that require attachment for feeding, fungi typically do not thrive in environments with low nutrients and organic matter (Richards et al., 2012). However, there may be important roles for saprotrophic fungi near the ocean floor where particulate matter settles into sediment (Richards et al., 2012) and on carbon-rich particulate matter (Bochdansky et al., 2017).

In marine environments, sinking particulate matter (aggregates) with a diameter greater than 0.5 mm is known as marine snow (Lampitt, 2001). Marine snow is a major carbon and energy source for organisms in the deep sea (Lampitt, 2001). These organic aggregates provide a unique microenvironment different from the surrounding waters (Duret et al., 2020). Diverse communities of microbes, including protozoans and bacteria are known to colonize marine snow (Simon et al., 2002), but knowledge on the fungal community associated with marine snow is limited

(Bochdansky et al., 2017; Duret et al., 2020). Marine snow contributes to the biological carbon pump which refers to the biological processes that transform atmospheric CO_2 into various forms of carbon and eventually sequesters large quantities of carbon to the deep ocean and abyssal sediments for long term storage (Herndl and Reinthaler, 2013) (Fig. 2). The gravitational settlement of marine snow is a major mechanism by which carbon is delivered from the surface to the deep sea (Lampitt, 2001), thus it is important to characterize the fungal community that is associated with marine snow and sinking particles.

The aim of this study was to characterize fungal communities during a spring bloom in a subarctic region near Hokkaido, Japan. The cruise track covered part of the western North Pacific subarctic region, which is known to be a highly productive area (Honda et al., 2017). I collected samples during two cruises conducted in March (beginning of the bloom) and May (end of the bloom) using the Marine Snow Catcher (MSC) (OSIL Inc., U.K.), which allows the collection of both the suspended and sinking particle samples in the same water body (McDonnell et al., 2015). Fungal communities in the suspended and sinking particle fractions were examined using 18S rRNA gene amplicon sequencing method (Banos et al., 2018). I used the fungal specific 18S rRNA primer FF390/FR1 (Vainio and Hantula, 2000) with blocking oligonucleotides to enhance the detection of marine fungal sequences (Banos et al., 2018).

To achieve my aim, I asked the following questions:

1) Are there fungal communities associated with sinking particles? If yes, are sinking particle-associated communities different from those suspended in seawater?

2) Are there differences in the fungal communities between the beginning (March) and the later stage (May) of the phytoplankton spring bloom?

3) Are there differences in fungal communities across different water layers with different environmental conditions?

Methods

Sample collection

Seawater samples were collected during two research cruises aboard the R/V *Shinsei Maru* in the Northern Pacific Ocean near Hokkaido. Cruise KS-21-4 was from March 11th to 20th, 2021 and cruise KS-21-7 was from May 3rd to 10th, 2021. Five stations were sampled in March and six in May (Fig. 3). At each station, samples were collected at three layers: the chlorophyll maximum layer (CM), the pycnocline (PC), and the bottom boundary layer (BBL). These layers were chosen because they represent vital points in a particle's vertical transport down to the deep ocean. The CM is the layer of high productivity, and it is where particles are formed. The PC marks a large increase in water density, thus creating a barrier where particles can accumulate. The BBL is near the seafloor, and it is where organic matter transformation takes place.

Two types of MSC, a small size (ca. 100 L in toral volume) and a large size (ca. 300 L), were used for collecting suspended and sinking particles (Fig. 4). The MSCs were washed with phosphate-free detergent prior to initial deployment. After sample collection at the target depth and subsequent retrieval, the MSC was set on board where it sat undisturbed for 2 hours to allow the sinking particles to settle to the bottom tray of the MSC. Afterwards, the suspended and sinking particles were collected from the upper part and the bottom tray of the MSC, respectively.

Suspended and sinking particle samples were vacuum filtered onto a 0.8 μm pore size cellulose acetate filter (47 mm diameter, Whatman). For suspended particle samples, 2 L of seawater was filtered. For sinking particle samples collected with the large MSC, approximately

500 mL of concentrated seawater was filtered. The volume of sinking particle samples collected with the large MSC differed between the KS-21-4 cruise (approximately 120 mL) and KS-21-7 cruise (approximately 180 mL) due to changes in the bottom collection tray. After filtration, samples were stored at –80 °C until further processing onshore.

Physicochemical data

Environmental data were obtained by other researchers on board and provided as the Cruise Data Summary from the cruise director (Prof. Toshi Nagata). Temperature and salinity were measured with a conductivity-temperature-depth device (SBE 911plus, Sea-bird Scientific). Nutrient concentrations were quantified with an auto-analyzer (AACS III, Bran+Luebbe). Chl a concentrations were determined using a fluorometer (Turner Designs).

DNA extraction and PCR amplification

Nucleic acids collected on cellulose acetate filters were extracted using the DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany) according to Hirai et al. (2017). Half of the frozen filter was cut into pieces smaller than 5 mm^2 on a sterile surface and placed into a 2 mL DNA LoBind tube (DNA LoBind® Tubes, Eppendorf、Hamburg, Germany). The sample was then lysed in 400 μL of FL Buffer (400 mM Tris-HCl [pH 8.0], 60 mM EDTA, 150 mM NaCl, and 1% [wt/v] SDS) at 60°C for 10 min. Next, 120 μL of 3M potassium acetate buffer (pH 4.8) was added to terminate the lysis reaction, followed by a 5 min incubation on ice. The sample was then centrifuged at 15,000 rpm at room temperature for 5 min and the supernatant product was transferred to a 1.5 mL DNA LoBind tube and centrifuged again. After collection of the supernatant, the supernatant was further purified using the DNeasy PowerSoil Kit. Following

extraction, DNA quantity was measured using the Quantus™ Fluorometer (Promega, Wisconsin, United States) and stored in a 1.5 mL DNA LoBind tube at –80 °C.

Illumina's "16S Metagenomic Sequencing Library Preparation" was used as a guideline for sequencing. Amplification of the 18S rRNA V7-V8 region was performed with the fungi-specific primer pair nu-SSU-1333-5'/nu-SSU-1647-3' (CGATAACGAACGAGACCT/ANCCATTCAATCGGTANT) (Vainio and Hantula, 2000). Along with blocking oligonucleotides to prevent co-amplification of non-fungal eukaryotic groups, this primer set was recently established as the most promising for sequencing marine fungal communities (Banos et al., 2018). The four blocking oligonucleotides with a 3'-amino linker C6 modification targeted the following groups: Stramenopiles (TCGCACCTACCGATTGAA), Alveolata (GTCGCTCCTACCGATTGA), Rhizaria (TTAA CGAACGAGACCTCGA), and *Telonema* (GACCTTAACC TACTAAATAGTTA) (Banos et al., 2018). PCR was performed three times for each sample and pooled before the first purification. The composition of reaction mixture for the first PCR was as follows: 0.4 μL of 2x Tks Gflex DNA Polymerase Low DNA and 9.4 μL of Gflex PCR Buffer (Takara Bio Inc., Shiga, Japan), 0.4 μL of each forward and reverse primers at a working concentration of 10 pmol/L, 0.4 μL of each of the four blocking oligonucleotides at a working concentration of 10 pmol/L. The volume of template DNA and distilled water (dH_2O) varied depending on the sample, such that each sample had 2-4 ng/20 μL of template DNA and the reagent volume totalled 20 μL. The PCR conditions were 94 °C for 2 min, 35 cycles of 94 °C for 30 s, 48 °C for 60 s, and 68 °C for 60 s, followed by a final extension at 68 °C for 10 min.

The resulting replicates from the first PCR were then pooled for a total of 60 μL and subjected to PCR clean-up with AMPure XP beads (Beckman Coulter Inc, California, United

States) following Illumina's protocol with slight modifications: i) 99 μL of AMPure XP beads were added to the PCR product at room temperature, ii) after the ethanol washes, the beads were dried for around 2 minutes, and iii) the beads were resuspended with 40 μL of dH_2O.

Indexing PCR was performed on the purified first PCR products to link the amplicons to indices (Nextera XT Index Kit v2 Set A). The reaction volumes for the index PCR were as follows: 2.5 μL of the first PCR products, 2.5 μL of each Primer 1 and Primer 2, 12.5 μL of 2x KAPA HiFi HotStart ReadyMix (Roche Holding AG, Basel, Switzerland), and 5 μL of dH_2O. The PCR conditions were as follows: 95 °C for 3 min, eight cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 30 s, and a final extension at 72 °C for 5 min.

Indexing PCR products were purified with AMPure XP beads similar to the first purification, but with the following modifications: i) 28 μL of AMPure XP beads was added to the PCR products, ii) after the ethanol washes (twice), the beads were dried for about 10 minutes, and iii) the beads were resuspended with 27.5 μL of dH_2O.

The cleaned-up DNA was measured and converted from ng/μL to nM with the following formula, where average library size was 399 bp.

$$\frac{\left(\text{concentration in}\frac{\text{ng}}{\mu\text{L}}\right)}{660\frac{\text{g}}{\text{mol}} \times \text{average library size}} \times 10^6 = \text{concentration in nM}$$

After the quantification of DNA concentration, samples with high DNA concentrations (>100 nM) were diluted with dH_2O. Then, DNA samples were added to the final library with a final concentration of 4 nM.

Library denaturation and dilution followed Illumina's protocol. After denaturation, the library and PhiX control were diluted to 10 pM and mixed at a ratio of 1:1. Then the library and

PhiX mixture was incubated at room temperature. Amplicons were sequenced using the Illumina MiSeq System with the Illumina MiSeq Reagent Kit v3 (600 cycles).

Sequence analysis/bioinformatics

Fastq files were imported into the QIIME 2 and all subsequent downstream processing was carried out on this platform (version 2021.11) (Bolyen et al., 2019). Cutadapt (version 3.5) (Martin, 2011) was used to remove primer sequences. Reads were then demultiplexed using the demux plugin on QIIME2. Forward and reverse reads were merged, quality control was performed, and chimeras were removed using the DADA2 algorithm (Callahan et al., 2016). Reads were truncated at 240 bp and 200 bp, for forward and reverse reads, respectively. Twenty bp were trimmed from both the forward and reverse reads. Amplicon sequence variants (ASVs) were generated and taxonomically classified with the SILVA 132 18S database at a sequence similarity of 99%.

Statistical analysis

Data processing and statistical analyses were performed on R (R Core team 2022) using the vegan (Oksanen et al. 2022) and phyloseq packages (McMurdie and Holmes, 2013), except that the taxonomic bar chart and pie chart were made using Excel (Microsoft, U.S.A.) and Spearman's correlation analysis was carried out using JMP Pro (SAS Institute Inc, U.S.A.). All ASVs were rarefied to the minimum of 32,174 reads. Fungal ASVs with less than one read across all samples (singletons) were discarded as they are likely sequencing artifacts (Tedersoo et al., 2010). Samples with only one fungal ASV occurrence were also discarded to allow for calculation of Bray-Curtis dissimilarity. Reads were transformed into relative abundances for visualization of the taxonomic bar plot and pie chart. Shannon and Simpson diversity indices were calculated and

visualized with boxplots. The Kruskal-Wallis test was used to assess significant differences of diversity indices between months (March and May), layers (CM, PC and BBL) and fractions (suspended and sinking fractions), followed by a post-hoc Dunn test with Bonferroni p-value adjustment. The nonmetric multidimensional scaling (NMDS) ordination and permutational analysis of variance (PERMANOVA) were conducted using the Bray-Curtis dissimilarity matrix. "adonis2" command was used for PERMANOVA.

Results

Environmental variables

In total, 66 samples were collected using the MSC at three layers (CM, PC, BBL) and two fractions (sinking and suspended) at different stations during the phytoplankton spring bloom (March and May 2021), with the exception of St.1 in May, where samples were only collected at the mesopelagic layer (Table 1). These samples were analyzed to investigate the variability of marine fungal communities across fraction, layer, and month.

Environmental variables at each station are summarized in Table 1. Temperature ranged from 0.48 °C to 3.92 °C in March and 1.49 °C to 8.11 °C in May, while salinity ranged from 32.25 to 34.69. Chl a concentration was measured in the CM and PC. The maximum chl a concentration was 11.03 μg/L, observed in March at St. 6 (in the CM layer), while the minimum chl a concentration of 0.20 μg/L was found in March at St. 5 (in the PC layer). Generally, chl a concentrations were higher at stations closer to the coast and decreased towards the open oceans.

Sequence performance and taxonomic composition of fungal community

Fungal sequences were detected in 58 samples (88% of samples) (Table 2). For two samples, extracted DNA concentration was too low to be included in the Illumina sequencing (Table 2). After rarefaction and removal of singletons, a total of 123, 076 reads were classified as fungi and clustered into 117 ASVs. Following the subsequent removal of samples with only one fungal ASV, 42 ASVs across 43 samples remained. Taxonomic affiliations of fungal ASVs are summarized in Table 3. Identified phyla included *Ascomycota* (6 ASVs), *Basidiomycota* (6 ASVs), *Chytridiomycota* (16 ASVs), *Cryptomycota* (3 ASVs), and *Zoopagomycota* (1 ASV). In terms of ASV number, 38% of ASVs belonged to *Chytridiomycota,* 14% to each of *Ascomycota* and *Basidiomycota*, 7% to *Cryptomycota*, 3% to *Zoopagomycota*, and 24% to unidentified fungi (Fig. 5). In terms of the relative abundance of reads, *Chytridiomycota* was the most abundant identifiable phylum (20%), followed by *Basidiomycota* (7%), *Ascomycota* (4%), *Cryptomycota* (1%), and *Zoopagomycota* (0.5%) (Fig. 6). Unidentified fungi accounted for 68% of total reads (Fig. 6).

Unique and common fungal ASVs across month, layer, and fraction

Venn diagrams were made to investigate whether there were ASVs uniquely associated with a particular month, layer, or fraction. 44% of ASVs were common between March and May, while 15% of ASVs were unique to March and 41% to May (Fig. 7A). The majority of ASVs (73%) were common between the sinking and suspended particle fractions (Fig. 7B). Between the three layers, only 12% of ASVs were shared, while 41% of the ASVs in the BBL were unique (Fig. 7C).

Alpha diversity of fungal community

To examine alpha diversity of fungal community, I calculated Shannon and Simpson diversity indices. Both diversity indices were higher in March than in May, although the difference was statistically insignificant (Fig 8). Both diversity indices were slightly higher for the suspended than sinking fraction, although there was no statistically significant difference between the two (Fig. 9). Between the sampling layers, Shannon and Simpson diversity indices were the highest in the BBL layer, with the difference between the CM and BBL being statistically significant for both the Shannon (Kruskal-Wallis, $P = 0.011$)(Dunn's test, $P = 0.0198$) and Simpson indices (Kruskal-Wallis, $P = 0.016$)(Dunn's test $P = 0.0241$) (Fig 10).

Variability of fungal communities across month, layer and fraction

Variability of fungal community composition was examined using NMDS ordination analysis. My results showed that samples from May and March form distinct clusters (Fig. 11). PERMANOVA results indicated that fungal community was significantly different between March and May ($P = 0.001$; Table 4). Fungal community was also significantly different across different layers (PERMANOVA, $P = 0.007$) (Table 4), with a cluster of BBL samples being noted on the NMDS plot (Fig. 12). The difference in fungal community between the sinking and suspended fractions was insignificant (PERMANOVA, $P = 0.191$) (Fig. 13, Table 4).

Occurrence pattern of prevalent fungal ASVs over months and across layers

The occurrence pattern of fungal ASVs across layer and month were examined using the abundance data of the ten most prevalent ASVs that occurred in more than 5 samples (Table 5, see also Table 3 for taxonomic affiliations of these ASVs). Three ASVs, including ASV2

(unidentified), ASV57 [*Chytridiomycota* (*Lobulomycetaceae*)] and ASV398 [*Ascomycota* (*Metschnikowiaceae*)] occurred in both March and May and across all layers (Table 5). In contrast, there were ASVs that occurred in a restricted month or layer (Table 5). These ASVs included four *Chytridiomycota* ASVs (ASV198, ASV293, ASV379, and ASV596) that occurred mostly in May, and three ASVs [ASV653 (*Chytridiomycota*), ASV693 (*Chytridiomycota*), ASV889 (unidentified)] that occurred mostly in the BBL. In contrast, three *Chytridiomycota* ASVs (ASV293, 379 and 596), belonging to *Rhizophydiales*, occurred mostly in CM and PC layers and not in the BBL. Spearman correlation analysis results showed that these *Rhizophydiales* ASVs were significantly positively correlated (Table 7). Another occurrence pattern of note is ASV379, which was almost exclusively found in the suspended fraction (Table 5).

Discussion

Fungal community in suspended and sinking particle fractions

Recently, marine sequencing surveys have revealed that fungi are ubiquitous in marine habitats (Amend et al., 2019). However, only a few studies have employed fungi-specific primers (Banos et al., 2020; Priest et al., 2021) and there is a knowledge gap regarding the diversity of marine fungi and their ecological roles. The present study used fungi specific primers along with blocking oligonucleotides (Banos et al., 2018), which was recently shown to be the most promising for marine fungi sequencing surveys (Banos et al., 2020; Priest et al., 2021). Using this new approach, I successfully detected fungal ASVs, which revealed novel features in fungal diversity and community structure during a phytoplankton bloom in the productive region of the western North Pacific Ocean. Indeed, to my knowledge, my study is the first fungi-targeted sequencing research conducted in this oceanic region where carbon export mediated by the biological carbon

pump is among the highest in the world oceans (Honda et al., 2017). I analyzed the fungal community in both suspended and sinking particle fractions. My results obtained using a Venn diagram and NMDS ordination (with PERMANOVA test) showed that the difference in fungal community composition between the two fractions was insignificant, with the majority of ASVs (73%) shared between the two fractions. My results are consistent with the previous study of Duret et al. (2020) who examined eukaryotic communities in suspended and sinking fractions using a eukaryotic universal primer in the Southern Ocean. They found that the variability of the fungal community between sinking and suspended fractions was less pronounced compared to that across sampling stations. However, our analysis of the prevalent ASV occurrence patterns also showed that one ASV (ASV379) tended to occur more frequently in suspended relative to sinking fractions (Table 6), suggesting that some selection processes might be involved in the colonization of fungi on marine snow. Although the mechanisms of the colonization by fungi on sinking particles requires investigation in future studies, my results showing the widespread occurrence of fungi on sinking particles suggest that fungi may play a role in the regulation of sinking carbon flux (biological carbon pump) in the oceans. For example, if parasitic fungi associated with marine snow destroy host cells (e.g. diatoms) and release small fragment of cell residues, this may result in the suppression of sinking carbon flux because smaller particles sink more slowly than larger particles. Reversely, if fungal hypha serves as a skeletal component of marine snow structures and strengthens the rigidity of marine snow (suppresses fragmentation), this may lead to the enhancement of sinking carbon flux. Future studies are clearly needed to investigate these possibilities.

Chytridiomycota was the most abundant phylum during the spring phytoplankton bloom in the subarctic waters

Overall, *Chytridiomycota* were the most abundant identified taxa in terms of ASV numbers (Fig. 5). In terms of read numbers of the identified fungi, *Chytridiomycota* also represented the most abundant group (Fig. 6). In fact, 7 out of 10 most prevalent ASVs in my samples belonged to the *Chytridiomycota* (Table 6). Previous studies conducted in marine environments have found that the *Dikarya* (*Ascomycota* and *Basidiomycota*) generally dominate fungal communities (de Vargas et al., 2015; Morales et al., 2019; Sen et al., 2021; Tisthammer et al., 2016). However, there is also increasing evidence indicating that the *Chytridiomycota* can be enriched in some coastal regions (Jeffries et al., 2016; Richards et al., 2015) and the Arctic Ocean (Comeau et al., 2016; Hassett et al., 2017; Kilias et al., 2020). Picard (2017) found that the *Chytridiomycota* were abundant in marine sediments rich in organic detritus. Members of the *Chytridiomycota* can act as both parasites and saprophytes (Van den Wyngaert et al., 2022), which may allow them to occupy a wide variety of ecological niches, especially in productive environments. The occurrence pattern of the prevalent ASVs in my study (Table 6) suggests the diversification of niche preference among *Chytridiomycota* ASVs. *Chytridiomycota* ASV57 (*Lobulomycetaceae*) displayed a generalist pattern and occurred in both March and May and across all layers (Table 5, Table 6). This result suggests that they occupied a broad range of environments in the studied region. Previous studies have found that *Lobulomycetaceae* include both saprophytes and parasites (Simmons et al., 2009) and are present in diverse environments, including coastal and oceanic environments, lakes and polar systems (Grossart et al., 2019). In contrast, there were *Chytridiomycota* ASVs that were specialists and occurred mostly in May (ASV198, ASV293, ASV379, and ASV596) or in BBL (ASV653, ASV693), suggesting that these ASVs adapt to more specific environments than ASV57

does. The three prevalent *Rhizophyliales* ASVs that occurred mostly in May and in CM and PC layers (ASV293, ASV379 and ASV596) were significantly positively correlated to each other (Table 7), suggesting that they have a similar niche-preference. *Rhizophydiales* have been reported to be parasites infecting diatoms (Garvetto et al., 2019) and dinoflagellates (Lepelletier et al., 2014) in marine environments.

Variations of fungal communities over months and across layers

NMDS ordination analysis and PERMANOVA results showed that there were temporal community differences (Fig. 11, Table 4). Previous studies have found seasonal variations in fungal community compositions in marine environments (Banos et al., 2020; Priest et al., 2021; Sun et al., 2017; Taylor and Cunliffe, 2016). As the spring bloom progresses, phytoplankton undergo succession (Needham and Fuhrman, 2016), and this could cause a change in parasitic fungal species, as the host (phytoplankton) community changes. Furthermore, different stages of the spring bloom favor different lifestyles. When living hosts are abundant, parasitic species would be common while nearing the end of the bloom, when dead organic matter is plentiful, then the saprotrophic lifestyle would be favored. However, as described in the previous section, I found that some ASVs that were unique to May (end of the bloom) included members of the *Chytridomycota* belonging to *Rhizophydiales*, which are known to infect diatoms and dinoflagellates (Fernández-Valero et al., 2022; Garvetto et al., 2019). Thus, fungal parasitism of phytoplankton might still be widespread even in May. Further investigation is required to clarify whether prevalent fungal trophic modes (saprotrophy vs parasitism) change over different stages of the spring bloom.

Samples were collected from three different oceanic layers with different environmental conditions, which may drive fungal community differences. Few ASVs were common among the three layers, while the BBL layer had a high number of unique ASVs (Fig. 7C). Morales et al. (2019) found that fungal communities in the epipelagic and mesopelagic were different. Tisthammer et al. (2019) found that benthic and pelagic fungal communities only share 15% of OTUs. Many of the BBL specific ASVs belonged to the *Chytridiomycota*, suggesting a role for saprophytic *Chytridiomycota* in the deep ocean.

High fungal diversity in the BBL

Alpha diversity indices (Shannon and Simpson) showed that diversity was high in the BBL relative to the CM (Fig. 10). To my knowledge, no previous study has reported on fungal diversity in the BBL of marine environments, and my result is the first to suggest that the BBL is a layer containing rich fungal communities. High fungal diversity in the BBL could partially be explained by the influence of sediment resuspension in this layer. Sediment resuspension, which is driven by hydrodynamic forcing (Hsu, 2016), may be a mechanism by which fungi that originated from sediments are introduced into the BBL layer. The addition of sediment-derived fungal species to the water column may enhance fungal diversity therein. In support of this hypothesis, previous studies have found that fungal diversity is high in sediments compared to the water column in marine environments (Picard, 2017; Richards et al., 2015). Trophic modes and fungal activity in the BBL should be examined in future studies.

Conclusion

My study is the first sequencing survey to examine fungal community compositions in suspended and sinking fractions collected in the western North Pacific region. Using recently developed fungi-specific 18S rRNA gene primer and blocking oligonucleotides (Banos et al., 2018), I successfully detected fungal ASVs from my samples. My results provide novel insights into fungal diversity and community variability across different water layers and over different stages of a spring phytoplankton bloom. The major findings include: 1) the widespread occurrence of fungi associated with sinking particles, suggesting that fungi potentially play a role in the regulation of carbon export, 2) *Chytridiomycota* ASVs were abundant members of the identified fungal phyla and displayed a range of occurrence patterns, supporting the emerging notion that understudied "basal fungal lineages" such as the *Chytridiomycota* are a potential key player in marine ecological processes (Ilicic and Grossart, 2022), 3) fungal communities differed between March (beginning of the bloom) and May (later stage of the bloom), suggesting that some fungi are exclusively closely associated with certain bloom stages, and 4) fungal diversity was high in the BBL, which could be explained by the introduction of sediment-associated fungi to the water layer near the ocean floor due to hydrodynamic forcing.

There are several limitations in my study, which should be resolved in future studies. Most importantly, a large fraction of fungal reads was unidentified even at the phylum level. This problem could be overcome in the future with the expansion of fungal sequence database especially for that of marine fungal communities. Another problem is that the rRNA gene sequencing does not provide metabolic information. The detected ASVs may be derived from less active or even dead fungal cells. In such a case, the role of these ASVs in biogeochemical cycles would be minimal. Also, it is often difficult to discuss trophic modes and ecological role of fungi from

phylogenetic data alone. Finally, read numbers are influenced by gene copy number, and may not be an accurate representation of the number of individuals in a sample. There is a large variation in genome size for fungi from under 10Mb to 177Mb (Mohanta and Bae, 2015) and this could introduce errors into the quantitative assessment of fungal abundance in marine environments.

Future directions to improve understanding require the addition of other techniques such as microscopy with fluorescence in situ hybridization combined with catalyzed reporter deposition to observe metabolically active target organisms (Priest et al., 2021), or functional gene surveys to gain insight into the ecological role that marine fungi may partake in. Another valuable, yet time-consuming option is isolating and culturing prominent, abundant ASVs, then conducting whole genome sequencing. The fungi-specific primer and blocking oligonucleotides had success in identifying more fungal ASVs, but classification to lower taxonomic levels was not possible, partially due to the short target amplicon sequence and insufficiencies in fungal sequence database. A combination of sequencing with additional primers that target other regions such as the ITS may yield better taxonomic resolution.

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Table 1: Environmental parameters compiled from the Cruise Data Summary of KS-21-4 and KS-21-7 cruises. Marine snow catcher type indicates the sampling volume the MSC (S, 100 l; L, 300 l). Layer abbreviations are as follows, CM is the chlorophyll maximum, PC is the pycnocline and BBL is the bottom boundary layer.

Cruise	Station	Depth (m)	Layer	MSC type	Salinity	Temperature (°C)	Chl a (µg/l)	NO_3 (µM)	NO_2 (µM)	NH_4 (µM)	PO_4 (µM)	$Si(OH)_4$ (µM)
KS214	2	20	CM	S	32.94	1.38	0.81	17.94	0.24	0	1.5	33.39
		150	PC	S	33.51	3.93	0.16	19.94	0.18	0	1.56	37.31
		1496	BBL	L	34.51	2.38		43.87	0.06	0.01	3.13	163.23
	3	30	CM	S	32.89	1.63	3.65	14.63	0.32	0.08	1.34	29.34
		250	PC	S	33.41	2.35	0.03	28.05	0.08	0	2.17	58.71
		1065	BBL	L	34.41	2.77		43.85	0.09	0.01	3.19	155.52
	4	10	CM	S	32.57	0.48	10.18	9.6	0.18	0.09	1.08	23.39
		70	PC	S	32.78	0.62	3.43	15.49	0.18	0.06	1.4	29.99
		301	BBL	L	33.47	3.38		19.32	0.23	0	1.62	37.47
	5	10	CM	S	32.61	0.95	10.54	9.4	0.24	0.12	1.04	20.29
		130	PC	S	33.06	1.81	0.2	18.65	0.24	0	1.55	36.23
		350	BBL	L	33.5	2.9		30.93	0.08	0	2.39	69.04
	6	10	CM	S	33.31	3.23	11.03	5.68	0.26	0.26	0.78	19.47
		25	PC	S	33.59	3.38	10.85	4.1	0.59	0.64	0.68	15.62
		81	BBL	L	33.71	3.53		7.17	0.33	1.44	0.91	20.84
KS217	1	300	300	S	33.68	5.54		38.4	0.03	0	2.82	81.21
		500	500	S	34.14	5.4		43.73	0.03	0	3.14	114.84
		1000	1000	L	34.4	3.04		44.2	0.04	0	3.16	147.99
	1.5	30	CM	S	33.02	4.53	1.62	9.3	0.16	1.05	1.03	2.95
		65	PC	S	33.09	2.67	0.76	23.73	0.4	1.47	2.03	41.12
		4344	BBL	L	34.69	1.49		37.07	0.07	0	2.56	150.21
	2	14	CM	S	33.4	8.11	1.05	2.6	0.09	0.08	0.33	4.55
		65	PC	S	33.49	5.52	0.17	12.03	0.19	0.56	1.04	17.95
		1505	BBL	L	34.52	2.34		43.3	0.04	0.07	3.16	159.85
	3	19	CM	S	32.7	5.04	0.87	0.15	0.06	0.05	0.31	0.17
		47	PC	S	33.55	6.74	0.49	8.91	0.21	1.34	0.93	11.27
		1082	BBL	L	34.42	2.75		42.73	0.14	0.12	3.24	149.81
	4	25	CM	S	32.25	3.3	8.02	3.34	0.2	0.62	0.62	2.15
		90	PC	S	33.18	1.93	0.52	20.48	0.27	0.63	1.77	36.57
		305	BBL	L	33.47	2.67		27.38	0.22	0.02	2.11	54.42
	5	22	CM	S	32.4	5.13		6.03	0.17	0.87	0.72	7.58
		52	PC	S	33.21	3.75	0.13	10.78	0.21	0.88	0.97	15.69
		361	BBL	L	33.72	3.3		35.61	0.1	0.26	2.71	81.21

Table 2: Overview of the sequencing performance and total reads and number of ASVs for each sample after DNA amplification and data processing. “Detection of fungal reads after sequencing” indicates whether fungal reads were detected (o) or not (x). Samples with NA were excluded from sequencing because insufficient amounts of DNA were obtained after the 1st PCR. “Detection of fungal reads after data processing” indicates whether fungal reads were present (o) or absent (x) after the removal of singletons and sample filtering (removal of samples with the occurrence of only one ASV; see Methods for explanation)

			Sinking				Suspended (NonS)			
Cruise	Station	Layer	Detection of fungal reads after sequencing	Detection of fungal reads after data processing	Total reads (rarefied)	Number of ASVs	Detection of fungal reads after sequencing	Detection of fungal reads after data processing	Total reads (rarefied)	Number of ASVs
KS214	2	CM	o	x	-	-	x	-	-	-
		PC	o	x	-	-	o	x	-	-
		BBL	x	-	-	-	o	o	28	3
	3	CM	x	-	-	-	o	x	-	-
		PC	x	-	-	-	o	x	-	-
		BBL	o	o	215	8	o	o	37	3
	4	CM	o	x	-	-	o	x	-	-
		PC	o	x	-	-	o	o	36	2
		BBL	o	o	23	3	o	o	71	5
	5	CM	o	x	-	-	o	o	90	4
		PC	o	o	48	2	o	o	118	4
		BBL	o	o	1427	13	o	o	284	8
	6	CM	o	o	25736	3	o	o	11893	2
		PC	o	o	7711	2	o	o	12093	2
		BBL	o	x	-	-	o	o	2029	2

Table 2 (continued)

KS217	1	300m(meso)	o	x	-	-	o	o	677	8
		500m(meso)	o	x	-	-	o	o	943	6
		1000m	x	-	-	-	o	x	-	-
	1.5	CM	o	o	17	2	o	o	220	2
		PC	o	x	-	-	o	o	235	2
		BBL	x	-	-	-	NA	-	-	-
	2	CM	o	o	144	3	o	o	177	4
		PC	o	x	-	-	o	o	282	5
		BBL	o	o	309	4	o	o	1060	7
	3	CM	o	o	98	4	o	o	1924	6
		PC	o	o	213	2	o	o	235	7
		BBL	o	o	1300	4	o	o	2148	6
	4	CM	o	o	1908	5	o	o	2755	6
		PC	NA	-	-	-	o	o	1770	5
		BBL	o	o	3731	5	o	o	3406	3
	5	CM	o	o	9491	5	o	o	5760	9
		PC	o	o	1031	5	o	o	4601	11
		BBL	o	o	696	9	o	o	1631	13

Table 3: Taxonomic affiliations of the fungal ASVs.

ASV ID	Kingdom	Subkingdom	Phylum	Subphylum	Class	Order	Family	Genus
ASV523	Fungi	*Dikarya*	*Ascomycota*	*Pezizomycotina*	*Dothideomycetes*	*Cladosporiales*	*Cladosporiaceae*	*Toxicocladosporium*
ASV2012	Fungi	*Dikarya*	*Ascomycota*	*Pezizomycotina*	*Leotiomycetes*			
ASV398	Fungi	*Dikarya*	*Ascomycota*	*Saccharomycotina*	*Saccharomycetes*	*Saccharomycetales*	*Metschnikowiaceae*	*Metschnikowia*
ASV1472	Fungi	*Dikarya*	*Ascomycota*	*Pezizomycotina*	*Dothideomycetes*	*Pleosporales*		
ASV954	Fungi	*Dikarya*	*Ascomycota*	*Pezizomycotina*	*Sordariomycetes*	*Hypocreales*	*Nectriaceae*	*Fusarium*
ASV1801	Fungi	*Dikarya*	*Ascomycota*	*Pezizomycotina*	*Eurotiomycetes*	*Eurotiales*	*Aspergillaceae*	*Aspergillus*
ASV1182	Fungi	*Dikarya*	*Basidiomycota*	*Ustilaginomycotina*	*Ustilaginomycetes*	*Ustilaginales*	*Ustilaginaceae*	*Melanopsichium*
ASV1082	Fungi	*Dikarya*	*Basidiomycota*	*Agaricomycotina*	*Agaricomycetes*	*Agaricales*	*Omphalotaceae*	
ASV540	Fungi	*Dikarya*	*Basidiomycota*	*Ustilaginomycotina*	*Malasseziomycetes*	*Malasseziales*	*Malasseziaceae*	*Malassezia*
ASV1935	Fungi	*Dikarya*	*Basidiomycota*	*Agaricomycotina*	*Agaricomycetes*	*Polyporales*		
ASV1617	Fungi	*Dikarya*	*Basidiomycota*	*Ustilaginomycotina*	*Malasseziomycetes*	*Malasseziales*	*Malasseziaceae*	*Malassezia*
ASV1348	Fungi	*Dikarya*	*Basidiomycota*	*Agaricomycotina*	*Tremellomycetes*	*Cystofilobasidiales*	*Mrakiaceae*	*Mrakia*
ASV57	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*	*Lobulomycetales*	*Lobulomycetaceae*	
ASV596	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*	*Rhizophydiales*		
ASV198	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*	*Rhizophydiales*		
ASV2432	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*			
ASV653	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*			
ASV693	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*	*Rhizophydiales*		
ASV2889	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*			
ASV379	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*	*Rhizophydiales*		
ASV1450	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*	*Rhizophydiales*		
ASV293	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*	*Rhizophydiales*	*Rhizophydiaceae*	
ASV1554	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*	*Rhizophydiales*	*Rhizophydiaceae*	
ASV1890	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*	*Rhizophydiales*	*Rhizophydiaceae*	
ASV1256	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*			
ASV2013	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*	*Rhizophydiales*		
ASV1782	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*			
ASV872	Fungi	-	*Chytridiomycota*	-	*Chytridiomycetes*	*Rhizophydiales*	*Rhizophydiaceae*	
ASV1095	Fungi	-	*Cryptomycota*	-	-	-	-	*Rozella*
ASV1733	Fungi	-	*Cryptomycota*					
ASV2508	Fungi	-	*Cryptomycota*	-	-	-	-	*LKM11 clade*
ASV325	Fungi	-	*Zoopagomycota*	*Entomophthoromycotina*	*Basidiobolomycetes*	*Basidiobolales*	*Basidiobolaceae*	*Basidiobolus*

Table 3 (continued)

ASV2	Fungi	Unidentified						
ASV889	Fungi	Unidentified						
ASV1490	Fungi	Unidentified						
ASV800	Fungi	Unidentified						
ASV1887	Fungi	Unidentified						
ASV1917	Fungi	Unidentified						
ASV854	Fungi	Unidentified						
ASV1772	Fungi	Unidentified						
ASV3439	Fungi	Unidentified						
ASV3518	Fungi	Unidentified						

Table 4: PERMANOVA results regarding community composition differences across month (March, KS-21-4 and May, KS-21-7), layer (CM, PC, BBL) and fraction (suspended and sinking).

	P-value	Pseudo-F ratio
Month	0.001	5.140
Layer	0.007	2.468
Fraction	0.191	1.453

Table 5: Distribution of the 10 most prevalent ASVs (values are read numbers after rarefaction). Refer to the legend in Fig. 5 for an explanation of the Sample Code.

Sample Code	Month	Station	Layer	Fraction	ASV2	ASV57	ASV398	ASV198	ASV889	ASV293	ASV379	ASV693	ASV596	ASV653
S214.S5.CM.NonS	March	5	CM	NonS	7	40	31	0	0	0	0	0	0	0
S214.S6.CM.NonS	March	6	CM	NonS	11738	155	0	0	0	0	0	0	0	0
S214.S6.CM.Sink	March	6	CM	Sink	25710	21	0	0	0	0	0	0	0	0
S214.S4.PC.NonS	March	4	PC	NonS	0	27	9	0	0	0	0	0	0	0
S214.S5.PC.NonS	March	5	PC	NonS	70	0	18	0	0	0	0	8	0	0
S214.S5.PC.Sink	March	5	PC	Sink	31	0	0	0	0	0	0	0	0	0
S214.S6.PC.NonS	March	6	PC	NonS	11765	328	0	0	0	0	0	0	0	0
S214.S6.PC.Sink	March	6	PC	Sink	7708	3	0	0	0	0	0	0	0	0
S214.S2.BBL.NonS	March	2	BBL	NonS	7	0	6	0	0	0	0	0	0	0
S214.S3.BBL.NonS	March	3	BBL	NonS	0	25	0	0	0	0	0	0	0	0
S214.S3.BBL.Sink	March	3	BBL	Sink	0	61	29	0	41	0	0	0	0	0
S214.S4.BBL.NonS	March	4	BBL	NonS	0	0	0	0	0	0	0	0	0	0
S214.S4.BBL.Sink	March	4	BBL	Sink	0	0	0	0	11	0	0	2	0	0
S214.S5.BBL.NonS	March	5	BBL	NonS	123	67	39	0	7	0	0	0	0	13
S214.S5.BBL.Sink	March	5	BBL	Sink	1136	0	57	6	69	0	0	67	0	0
S214.S6.BBL.NonS	March	6	BBL	NonS	1922	107	0	0	0	0	0	0	0	0

Table 5 (continued)

S217.S1.5.CM.NonS	217	3	0	0	0	0	0	0	0	0
S217.S1.5.CM.Sink	0	10	0	0	0	0	0	0	0	0
S217.S2.CM.NonS	151	11	7	0	0	0	0	0	0	0
S217.S2.CM.Sink	15	0	0	0	0	0	0	0	0	0
S217.S3.CM.NonS	1420	0	0	397	0	18	48	0	37	0
S217.S3.CM.Sink	71	0	0	0	0	0	4	0	10	0
S217.S4.CM.NonS	2078	632	0	27	0	0	0	0	0	0
S217.S4.CM.Sink	1818	72	0	0	0	0	0	0	0	0
S217.S5.CM.NonS	5232	285	0	17	0	103	67	0	1	0
S217.S5.CM.Sink	9358	61	0	0	0	60	8	0	0	0
S217.S1.5.PC.NonS	210	25	0	0	0	0	0	0	0	0
S217.S2.PC.NonS	248	16	1	8	0	0	0	0	0	0
S217.S3.PC.NonS	123	12	49	0	0	0	10	0	0	0
S217.S3.PC.Sink	210	0	0	3	0	0	0	0	0	0
S217.S4.PC.NonS	1521	218	0	17	0	0	0	0	0	9
S217.S5.PC.NonS	3624	832	10	0	0	53	49	0	7	0
S217.S5.PC.Sink	903	113	0	0	0	1	0	0	11	0
S217.S2.BBL.NonS	636	349	0	0	0	0	0	0	0	9
S217.S2.BBL.Sink	82	215	6	0	0	0	0	0	0	0
S217.S3.BBL.NonS	1735	332	0	0	0	2	0	0	0	0
S217.S3.BBL.Sink	1088	194	0	0	0	0	0	7	0	0
S217.S4.BBL.NonS	1869	1372	0	165	0	0	0	0	0	0
S217.S4.BBL.Sink	3346	263	0	112	0	0	0	0	0	0
S217.S5.BBL.NonS	1193	207	17	9	9	0	0	29	0	125
S217.S5.BBL.Sink	238	373	0	0	6	0	0	22	0	23

Table 6: Occurrence pattern of the 10 most prevalent fungal ASVs. Specific category for the month (March or May), layer (CM, PC or BBL) or fraction (suspended or sinking) is indicated if reads number in that category accounts for >90% of the total reads. For example, the read number of ASV198 in May is 755, which accounts for 99% (value in the parenthesis) of total reads for this ASV.

Occurrence pattern	ASV ID	Phylogenetic affiliation	Abundance (total reads)	Month	Layer	Fraction
Generalist	ASV2	Unidentified	97603			
	ASV57	*Chytridiomycota* (*Lobulomycetaceae*)	6429			
	ASV398	*Dikarya* (*Metschnikowia sp*)	279			
Specialist (May)	ASV198	*Chytridiomycota* (*Rhizophydiales*)	761	May (99%)		
	ASV293	*Chytridiomycota* (*Rhizophydiales*)	237	May (100%)	CM&PC (99%)	
	ASV379	*Chytridiomycota* (*Rhizophydiales*)	186	May (100%)	CM&PC (100%)	Suspended (94%)
	ASV596	*Chytridiomycota* (*Rhizophydiales*)	66	May (100%)	CM&PC (100%)	
Specialist (BBL)	ASV653	*Chytridiomycota* (*Chytridiomycetes*)	179	May (93%)	BBL (95%)	
	ASV693	*Chytridiomycota* (*Rhizophydiales*)	135		BBL (94%)	
	ASV889	Unidentified	143		BBL (100%)	

Table 7: Spearman's correlation among the prevalent fungal ASVs (ASVs that occurred in more than 5 samples) listed in Table 5. The correlation coefficients indicated with bold values in colored cells (orange, positive; blue, negative) were significant (P<0.05) after the Benjamini-Hochberg correction for multiple comparison.

	ASV596	ASV379	ASV293	ASV889	ASV2	ASV398	ASV57	ASV198	ASV653
ASV379	**0.697**								
ASV293	**0.701**	**0.6185**							
ASV889	-0.151	-0.168	-0.168						
ASV2	-0.026	-0.019	0.046	**-0.4974**					
ASV398	-0.11	0.019	-0.13	0.105	-0.413				
ASV57	-0.044	-0.065	0.076	-0.086	-0.418	0.189			
ASV198	0.165	0.126	0.141	-0.022	0.002	-0.061	0.065		
ASV653	-0.108	-0.12	-0.12	0.32	-0.187	0.062	0.345	0.141	
ASV693	-0.12	-0.133	-0.133	0.431	-0.214	0.14	-0.038	0.084	0.287

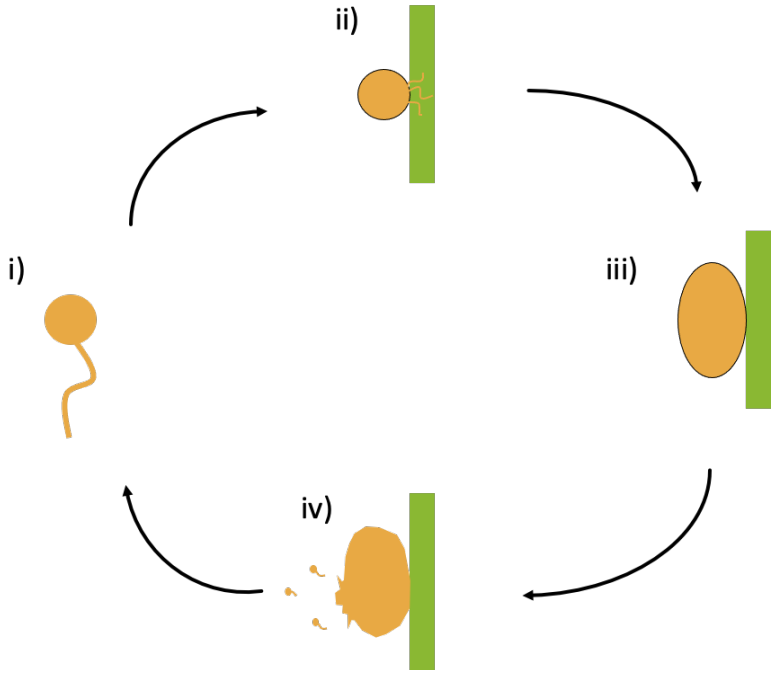


Figure 1: Asexual life cycle of *Chytridiomycota*. i) zoospore searching for host ii) zoospore attaches to host and anchors itself with rhizoids to siphon host nutrients iii) zoospore grows and matures into zoosporangium where zoospores mature iv) zoospores are released and look for another host.

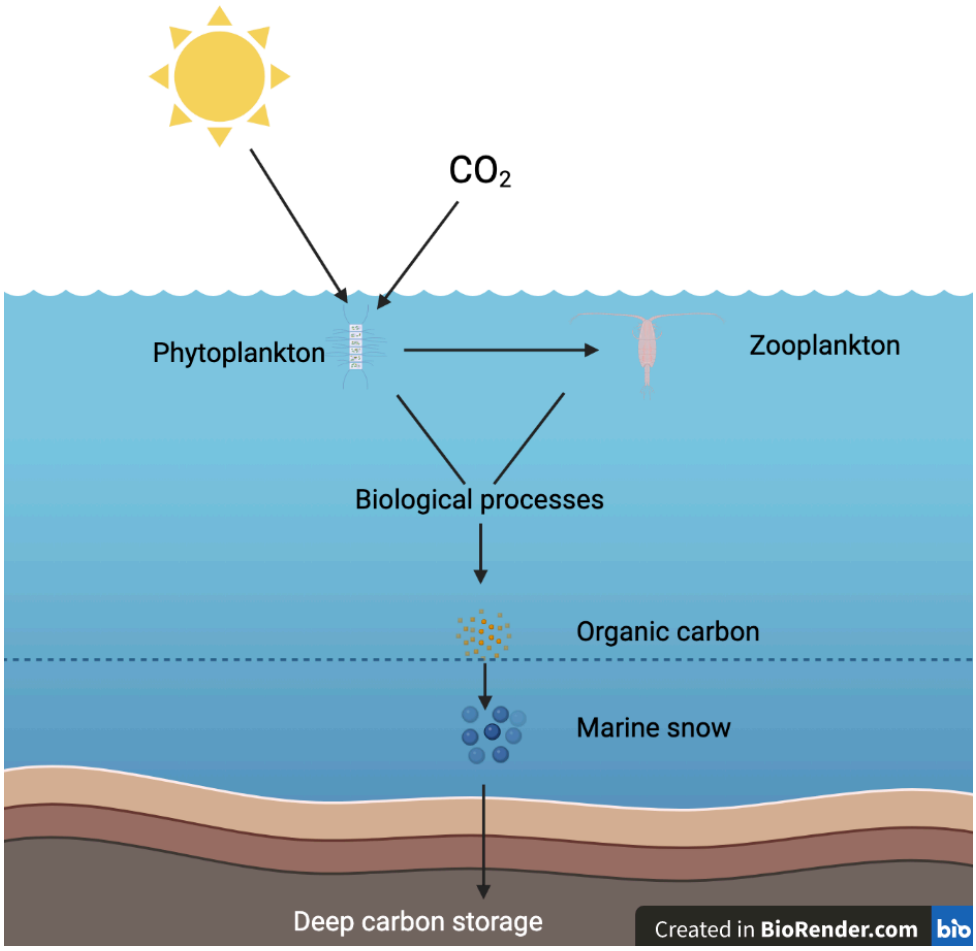
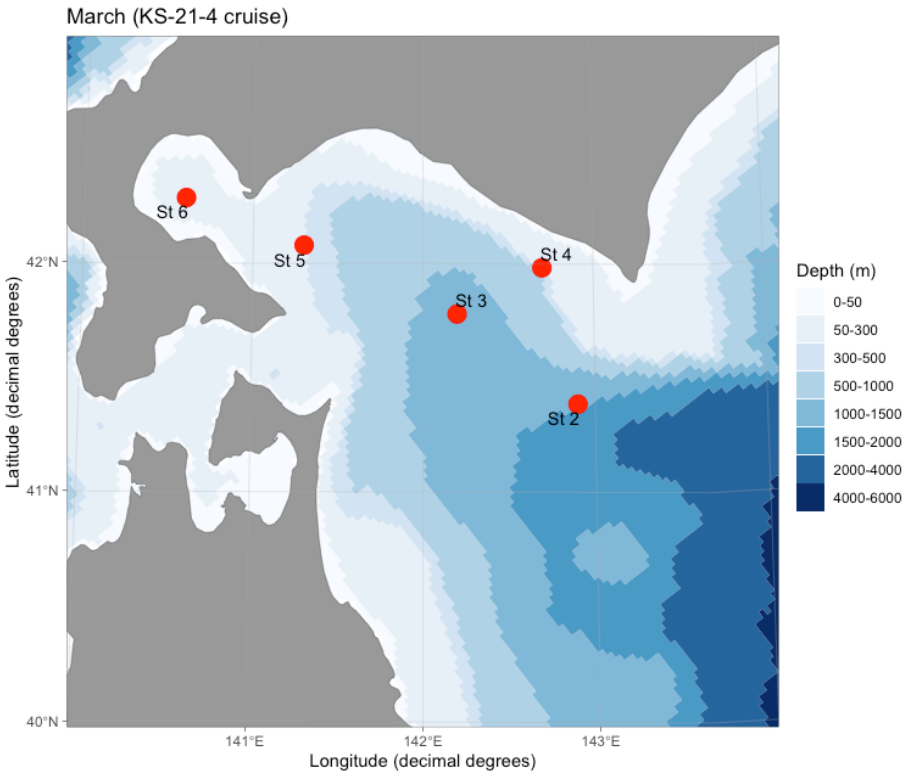


Figure 2: Carbon sequestration in marine ecosystems driven by the gravitational settlement of marine snow. This process is known as the biological carbon pump and it drives carbon storage in the deep sea and sediments. Clarifying the biological processes involved in the formation, transformation, and decomposition of marine snow is important for a better understanding of the regulation of the biological carbon pump. The image was made using BioRenderer (https://biorender.com/)

A



B

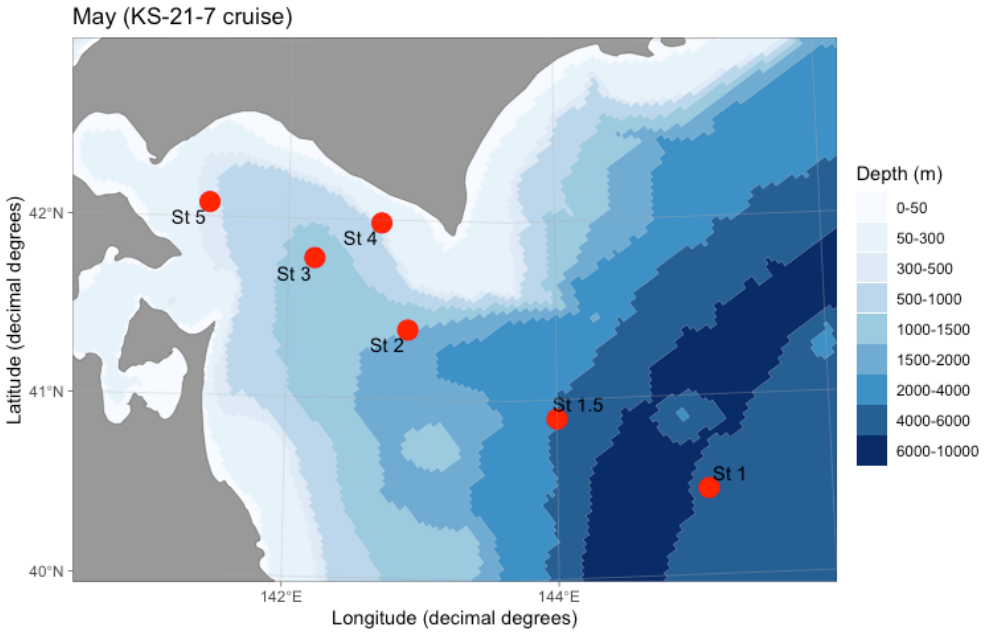


Figure 3: Sampling stations deployed during (A) the March cruise (KS-21-4) and (B) the May cruise (KS-21-7). The map was made using ggOceanMaps. (https://mikkovihtakari.github.io/ggOceanMaps/)

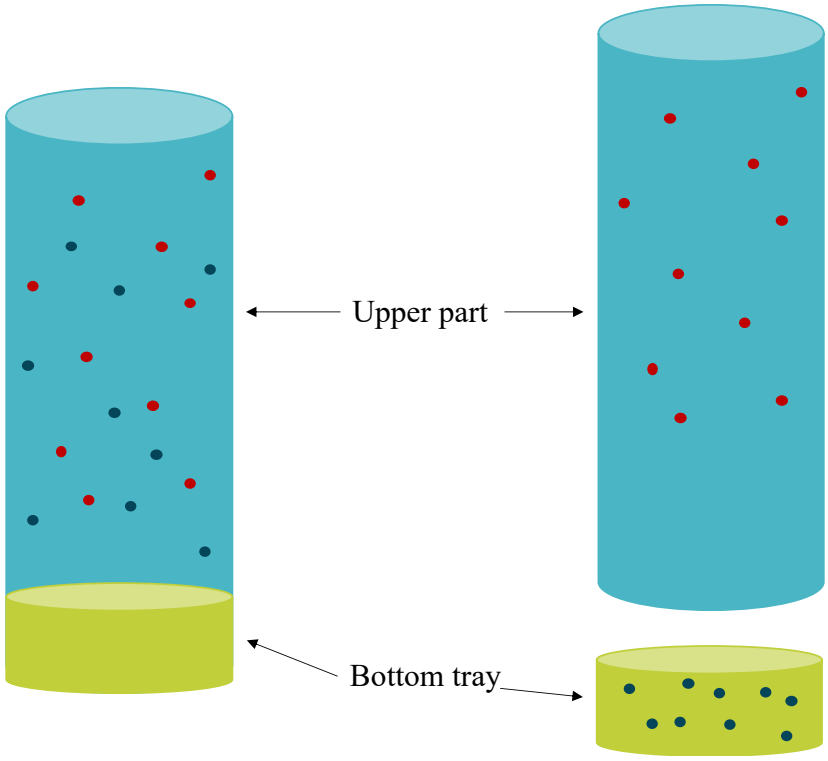


Figure 4: Simplified schematic of the MSC. The left is immediately after retrieval, when suspended and sinking particles are mixed. The right is after the MSC sits for 2 hours, to allow for sinking particles to settle. Red circles represent suspended particles and blue circles represent sinking particles. Suspended particle fraction was collected from the upper part of the MSC, whereas sinking particle fraction was collected from the bottom tray.

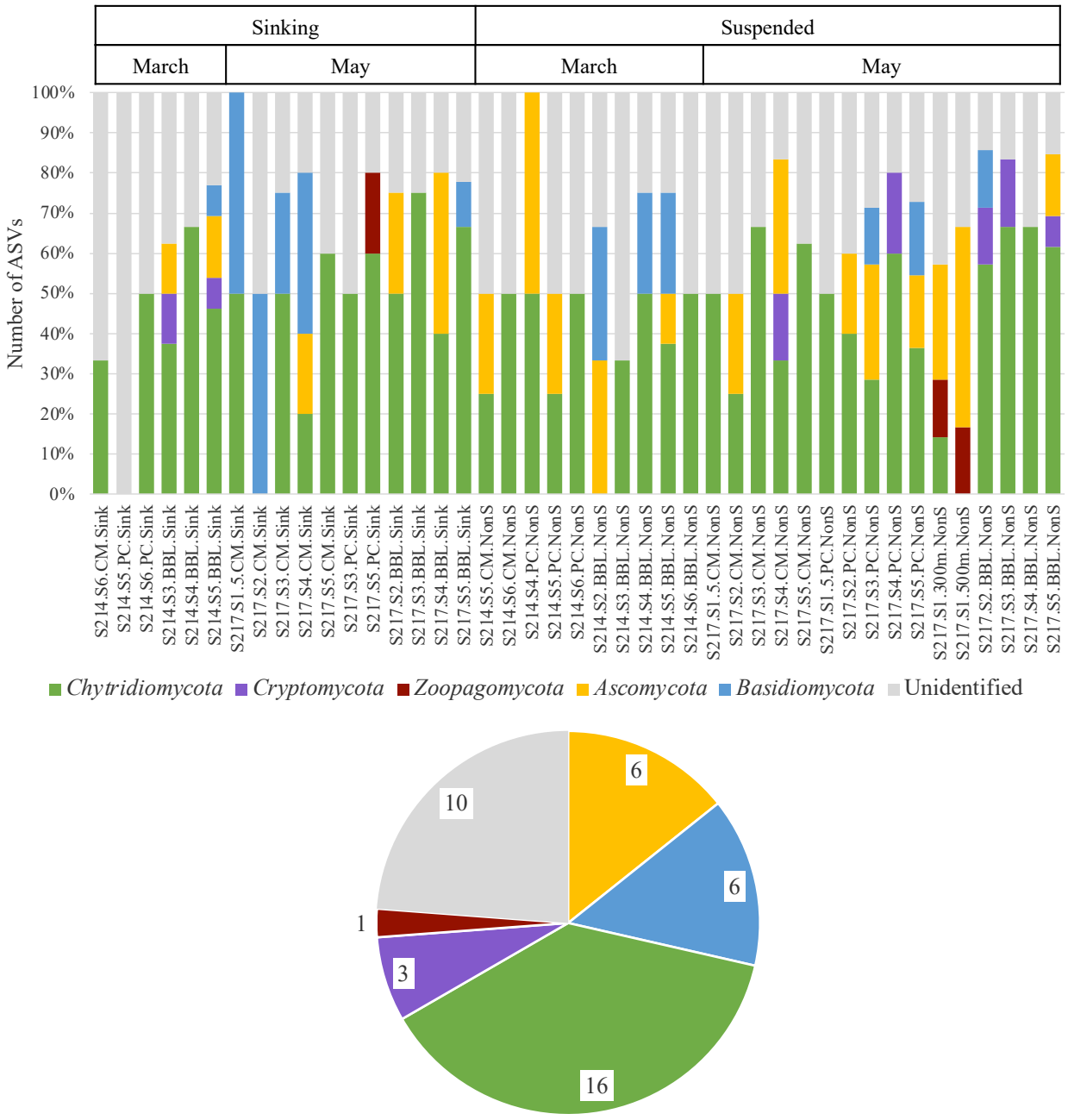


Figure 5. Phylum level composition of fungal communities in terms of ASV numbers. Sample codes indicate month (S214 and S217 which indicate the cruises conducted in March and May, respectively), sampling station (from S1 to S6, see Fig. 3), sampling layer (CM, PC, or BBL. Mesopelagic samples are indicated using the depth layers including 300 m and 500 m), and fraction (NonS, suspended fraction; Sink, sinking fraction). ASV's belonging to the *Chytridomycota* represented 38% of total ASV's.

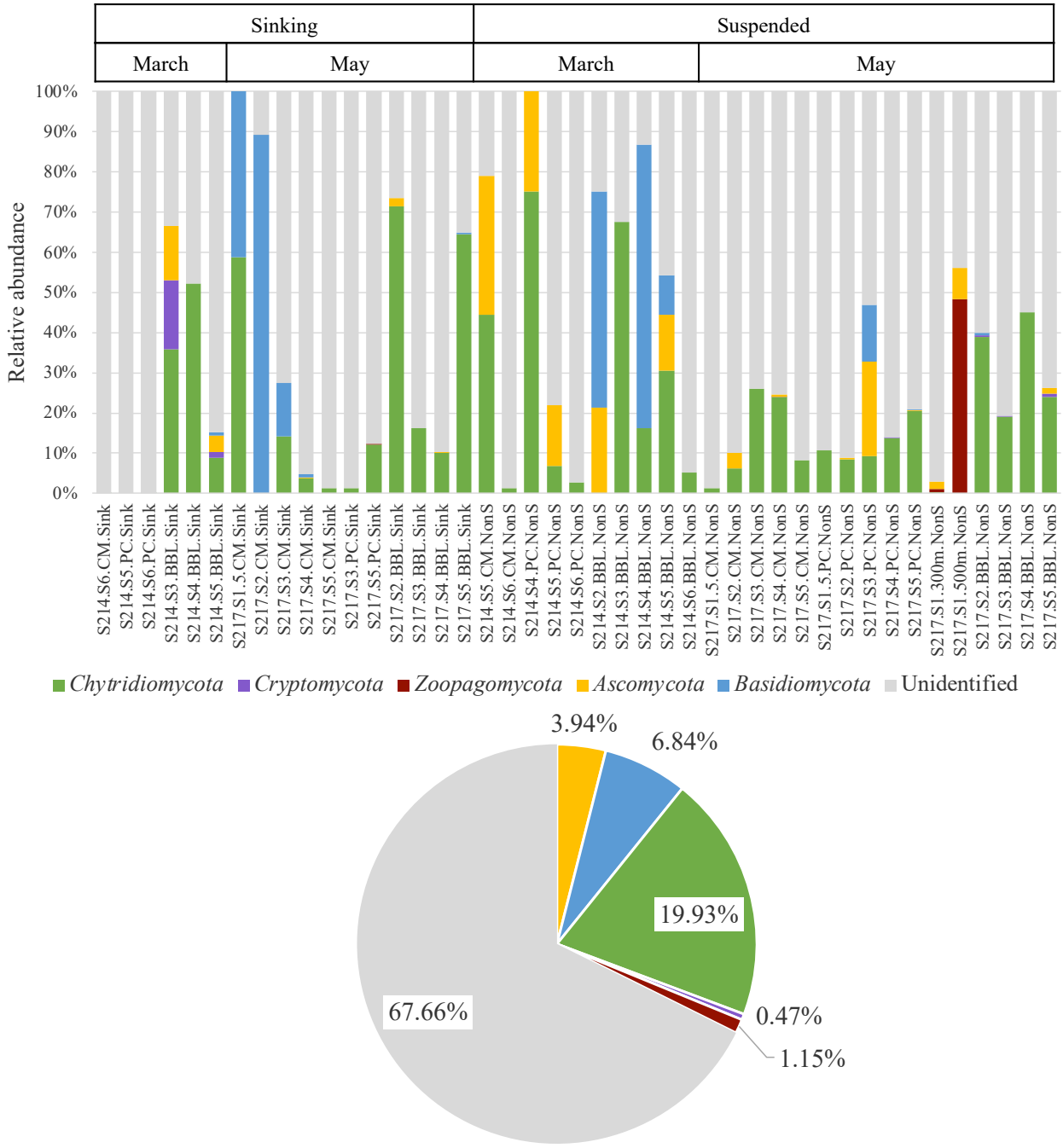


Figure 6: Phylum level composition of fungal communities in terms of ASV abundance (relative fungal read abundance). See the legend of Figure 5 for the explanation of sample codes. *Chytridiomycota* were the most prevalent identified phyla, accounting for 20% of total fungal reads.

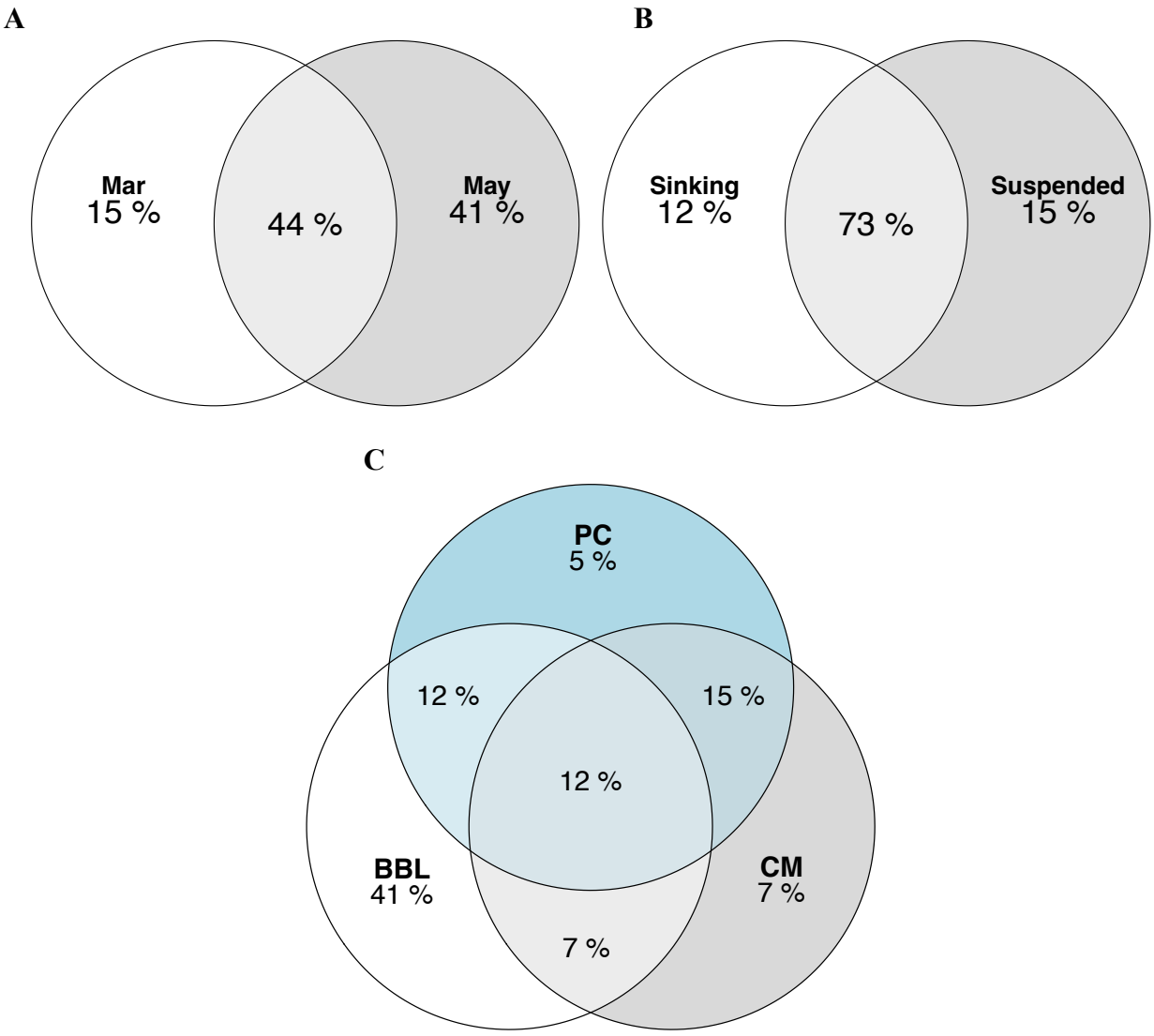


Figure 7: Venn diagrams to indicate the percentages of shared and unique ASVs. A) Comparison between month, B) fraction, and C) layer.

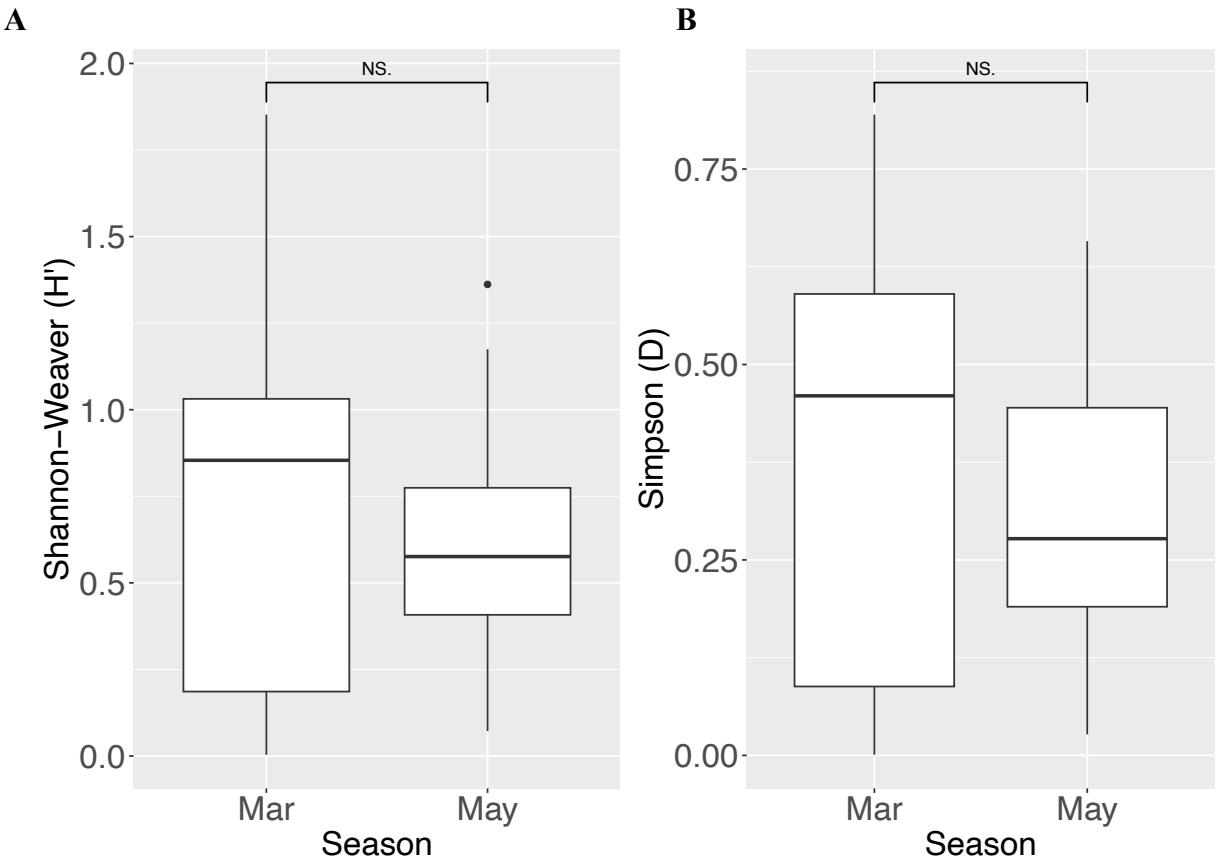


Figure 8: Diversity indices calculated for each month (March and May). Boxes represent the interquartile range between the first and third quartiles and the horizontal line inside is the median. Whiskers represent the maximum and minimum. Points outside of the whiskers are outliers. A) Shannon diversity; B) Simpson diversity.

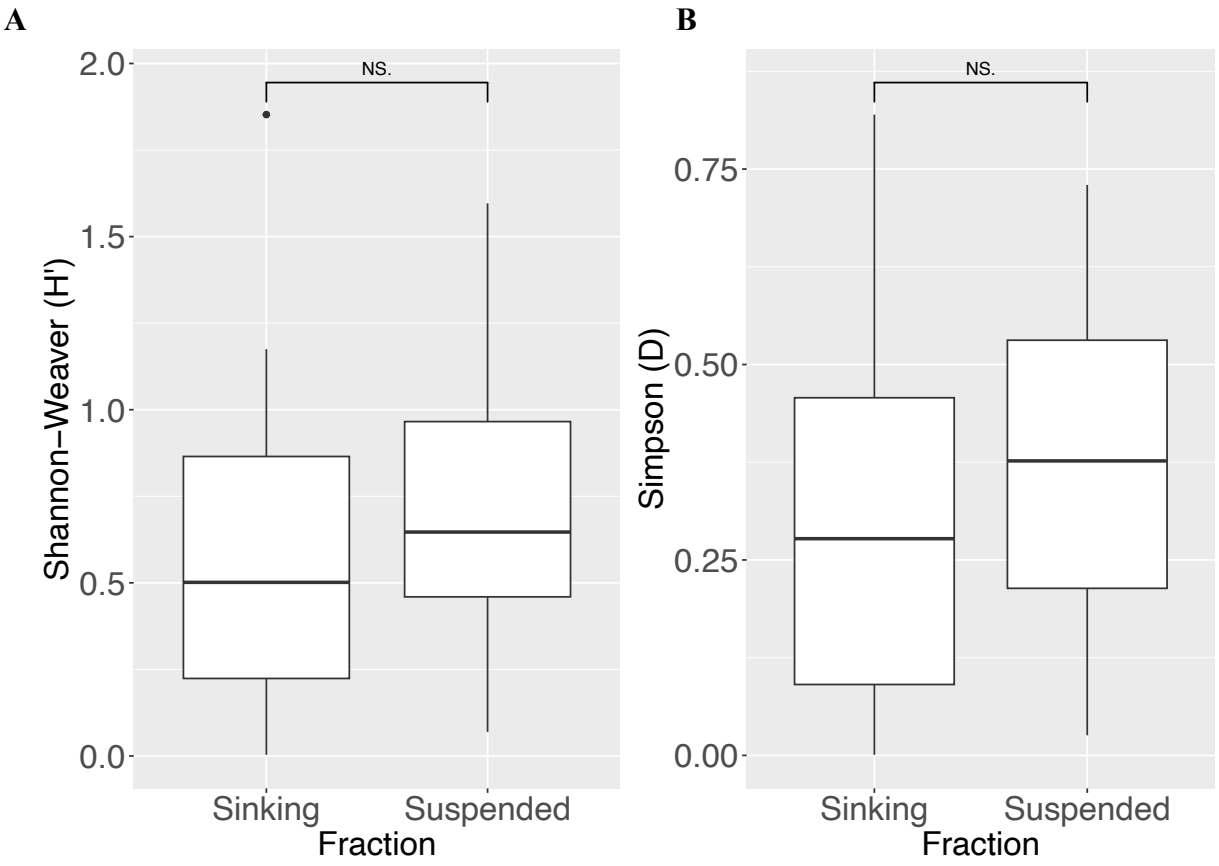


Figure 9: Diversity indices calculated for each fraction (suspended and sinking). Boxes represent the interquartile range between the first and third quartiles and the horizontal line inside is the median. Whiskers represent the maximum and minimum. Points outside of the whiskers are outliers. A) Shannon diversity; B) Simpson diversity.

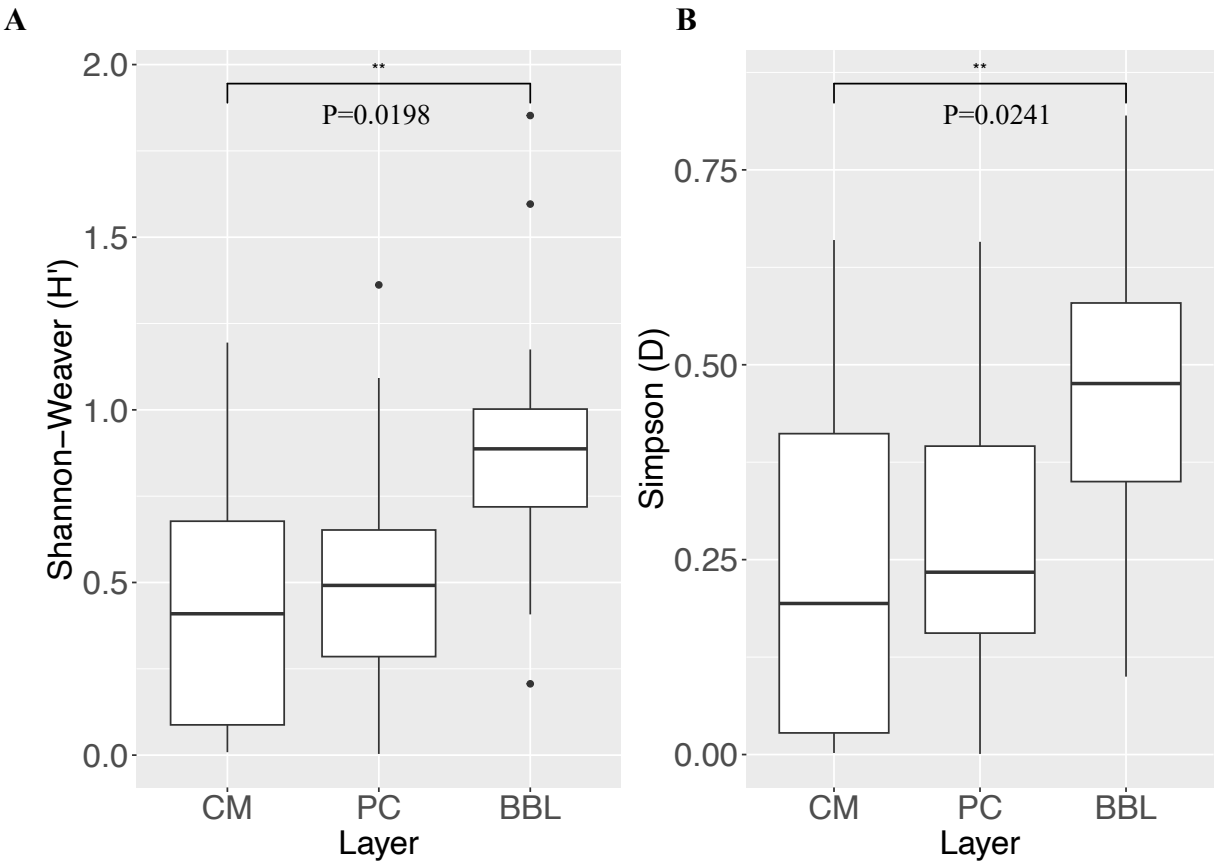


Figure 10: Figure 10: Diversity indices calculated for each layer (CM, PC and BBL). Boxes represent the interquartile range between the first and third quartiles and the horizontal line inside is the median. Whiskers represent the maximum and minimum. Points outside of the whiskers are outliers. A) Shannon diversity; B) Simpson diversity. Diversity indices at BBL were significantly larger than those at CM.

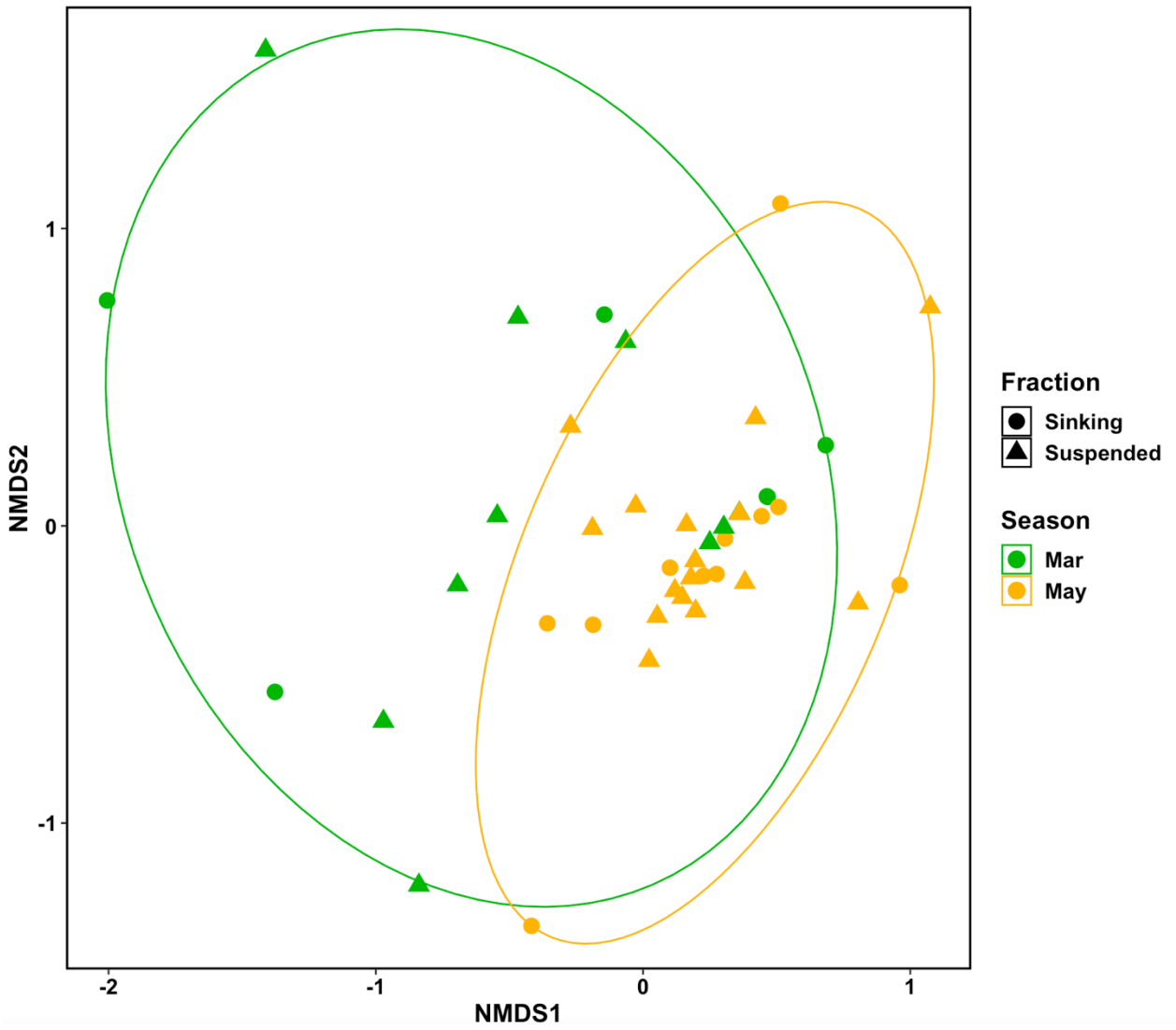


Figure 11: NMDS plot calculated using the Bray-Curtis dissimilarity. Stress value is 0.1. Colour coded for months (March and May).

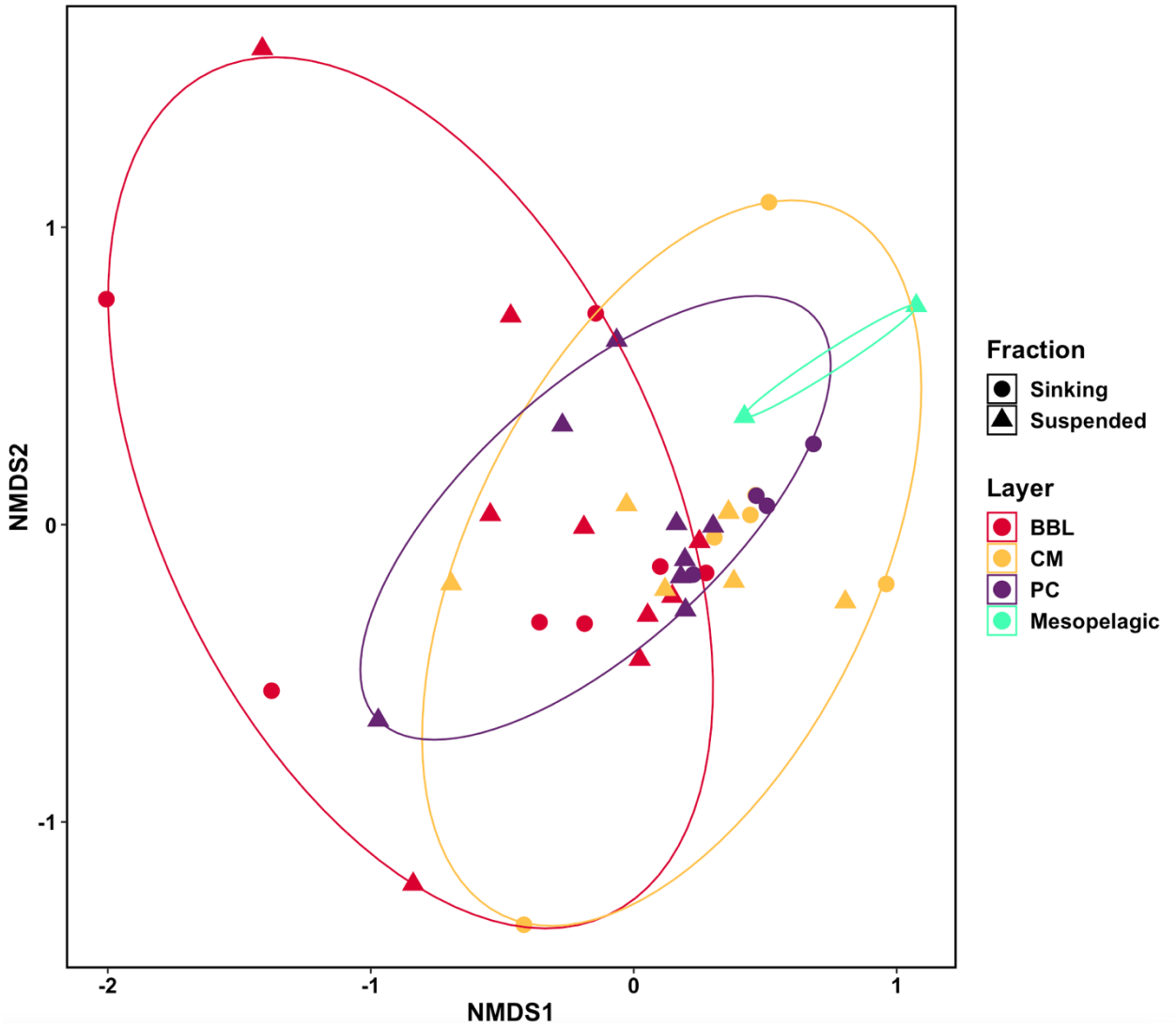


Figure 12: NMDS plot calculated using the Bray-Curtis dissimilarity. Stress value is 0.1. Colour coded for layers (CM, PC, BBL and mesopelagic).

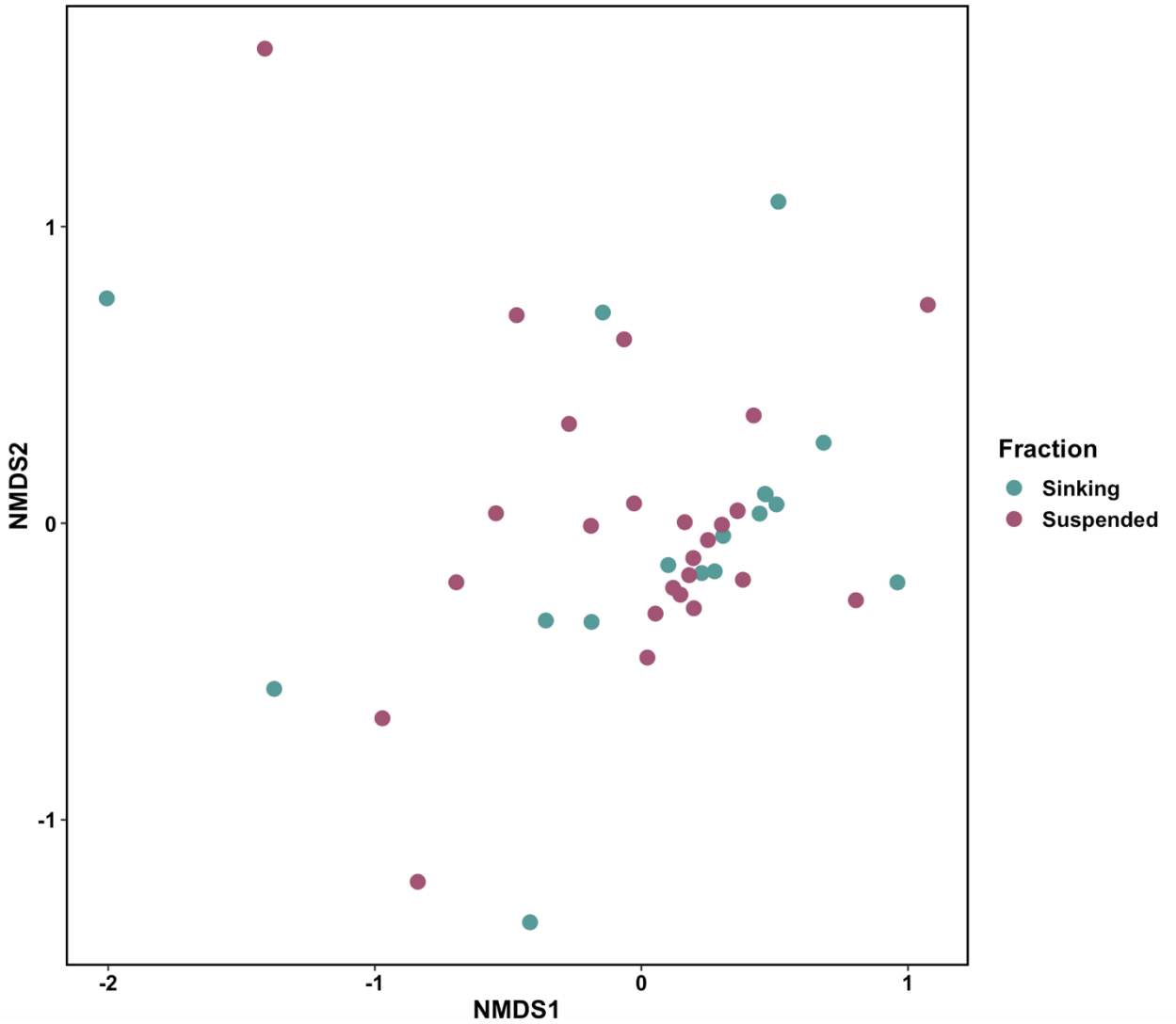


Figure 13: NMDS plot calculated using the Bray-Curtis dissimilarity. Stress value is 0.1. Colour coded for fractions (suspended and sinking).