Fungal community dynamics associated with harmful cyanobacterial blooms in two Great Lakes

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Abstract

Harmful algal blooms (HABs) impose major costs on aquatic ecosystems worldwide, including the Laurentian Great Lakes. Microbial consumers, including fungi, can have important interactions with bloom-forming algae and cyanobacteria, although relatively few studies have investigated the relationship between fungi and HABs. We examined changes in the aquatic fungal community coincident with the occurrence of large cyanobacterial blooms in two areas of the Great Lakes (western Lake Erie and Saginaw Bay, Lake Huron). We collected water samples over the course of bloom development, peak, and decline from 3 sites in western Lake Erie on 11 dates and 2 sites in Saginaw Bay on 4 dates. Single molecule sequencing (PacBio RS II) with two molecular markers (the internal transcribed spacer (ITS) of the rRNA locus using fungal-specific primers and the 18S rRNA with primers targeting early-diverging lineages of fungi) was used to estimate fungal community composition. Results indicate a diverse fungal community within the lakes, including several major fungal phyla. The Chytridiomycota were particularly well-represented (54.8% and 45.4% of ITS and 18S sequences, respectively), and we also found representation from both Cryptomycota and Aphelidiomycota, which are putatively obligate intracellular parasites. Further, we found associations between the fungal community (alpha diversity; community composition) and measures of bloom magnitude (chlorophyll a, phycocyanin, and microcystin concentrations) in western Lake Erie. Our results suggest potentially important spatial and temporal heterogeneity in the fungal community that motivates further research on functional importance of fungi in the Great Lakes and consequences for HABs and freshwater ecosystems more broadly.

Keywords: harmful algal blooms (HABs), *Microcystis aeruginosa*, Chytridiomycota, Cryptomycota, Aphelidiomycota

Introduction

Harmful algal blooms (HABs) impose major ecological and economic costs in aquatic ecosystems worldwide (Dodds et al., 2009; Hallegraeff, 1993). For instance, in portions of the Laurentian Great Lakes, HABs dominated by the cyanobacterium, *Microcystis aeruginosa*, are common, such as in western Lake Erie, Saginaw Bay in Lake Huron, or Green Bay in Lake Michigan (Sayers et al., 2016), with impacts on drinking water quality, recreational use, and the local economy (Steffen et al., 2014; Watson et al., 2016). Of particular concern, HABs have increased in western Lake Erie in recent years (Bridgeman et al., 2013; Sayers et al., 2016), in contrast to other locations, such as Saginaw Bay, where bloom magnitude has not increased substantially (Wynn et al., 2021). Climatic factors, selective feeding by dreissenid mussel invasion, and changes in nutrient availability are believed to be the primary driving factors behind record blooms in western Lake Erie (Michalak et al., 2013; Vanderploeg et al., 2001). However, other factors, such as natural enemies, may also influence bloom magnitude (Van Wichelen et al., 2016) and have not been fully explored.

For example, microbial consumers, including parasites, can influence the algal population dynamics (Bruning et al., 1992; Gerphagnon et al., 2013; Ibelings et al., 2011) and thereby the extent of algal blooms (Sigee et al., 1999; Smith and Schindler, 2009). One such group is aquatic fungal parasites, by which large fractions of cyanobacteria or other phytoplankton host cells can be parasitized (Gerphagnon et al., 2017, 2015, 2013; Gleason et al., 2015). Previous studies using molecular data have documented major shifts in the fungal community associated with HABs, particularly in Lake Taihu in China (Cai et al., 2018; Chen et al., 2010; Zhang et al., 2018). Such shifts may reflect changes in abundances of parasitic fungi interacting with blooms and also changes in the fungal community due to variation over time in the amount and composition of organic matter resulting from the blooms. However, the identity and functional importance of many fungi in aquatic systems and related to HABs particularly, including in the Great Lakes, is poorly understood. Assessing the influence of important but understudied taxa like the fungi in the Great Lakes is particularly pressing given large recent changes in environmental factors, such as climate (Michalak et al., 2013) and nutrient loading (Bosch et al., 2014), that may influence HABs dynamics and algal-pathogen interactions (Ibelings et al., 2004; Gleason et al., 2015).

The kingdom Fungi is comprised of 12 phyla of diverse ecosystem role and symbiotic interactions. In aquatic ecosystems, both Ascomycota and the zoospore producing Chytridiomycota are major components. The Chytridiomycota are of particular interest because they are known to be the major group of fungi that parasitize algae (Frenken et al., 2017; Kagami et al., 2007) and may influence to dynamics of algal blooms (Gerphagnon et al., 2017; Sime-Ngando, 2012), such as in the diatom *Asterionella* (Ibelings et al., 2011). In industrial production of algae, chytrids and another group of early-diverging fungi called aphelids (Aphelidiomycota) are also associated with rapid collapse of algal monocultures (Letcher et al., 2013; Longcore et al., 2020). Some chytrids are documented from both *Microcystis* (Sen, 1988) and other cyanobacteria (Gerphagnon et al., 2013; McKindles et al., 2020). While observations of infection in *Microcystis* by chytrids are relatively rare, there is no study that has tested whether chytrids respond to *Microcystis* blooms. Further, fungi may play an important role in decomposition of algal biomass produced during large blooms (Grossart et al., 2019). We therefore hypothesized that fungal communities associated with blooms change due to either (1) increases in the abundance of fungi that parasitize bloom-forming taxa and/or (2) shifts in organic matter availability and composition during bloom progression that impact the fungal community.

As a first step toward understanding the influence of fungal pathogens on HABs in the Great Lakes, we sought to evaluate the changes in the fungal community associated with blooms of *M. aeruginosa* in western Lake Erie and Saginaw Bay using metabarcoding of communities with DNA sequencing (Jobard et al., 2012; Monchy et al., 2011). Both lakes were sampled to allow a comparison between Lake Erie, which has experienced recent increases in bloom magnitude, and Saginaw Bay, which has not experienced similar changes (Wynne et al., 2021). We collected water samples over the course of bloom development, peak, and subsequent decline during the summer and early fall of 2014 and assessed the relative abundance of different fungi using single molecule PacBio sequencing. We broadly surveyed our samples for potentially important parasites of problematic algae species, including chytrids, and also members of the phyla Cryptomycota and Aphelidiomycota, recently described groups of early-diverging fungi, known primarily through environmental DNA sequences from aquatic and marine habitats (Jones et al., 2011; Karpov et al., 2014). Use of molecular approaches allowed us to explore the presence of many fungi that are not readily cultured but of potential functional importance for bloom dynamics and major ecosystem functions (e.g., nutrient cycling, decomposition).

Methods

Sample Collection

Depth-integrated whole water samples were collected from a boat via peristaltic pump between June 16th and October 27th, 2014 from three sites (WE2, WE4 and WE12) in the western basin of Lake Erie on 11 dates (approximately every two weeks) and between June 26th and September 23rd from two sites in Saginaw Bay (SB2 and SB14), Lake Huron, on 4 dates (approximately monthly). These sites have been monitored long-term by the NOAA Great Lakes Environmental Research Laboratory (GLERL) and the Cooperative Institute for Great Lakes Research (CIGLR); WE12 is near the water intake for the city of Toledo, which experienced a temporary drinking water ban due to a HAB during the summer of 2014, WE2 is close to where the Maumee River enters the lake, and WE4 is offshore and tended to be closer to the edge of the bloom. In Saginaw Bay, SB2 is nearshore while SB14 is more offshore. We collected samples concurrently with other samples collected by NOAA-GLERL and other researchers to assess bacterial communities (Berry et al., 2017) and water quality (e.g., pH, temperature, conductivity, and concentrations of algal pigments and cyanotoxins). Water samples were collected into 20 L polypropylene carboys (Erie) or 4 L sample bottles (Saginaw Bay); samples were transported to the laboratory and immediately processed by filtering sequentially through 100-micron mesh, 20-micron mesh, and then 0.8 micron filters, resulting in three sample size fractions (>100 micron, 20-100 micron, 0.8-20 micron). This study includes data for two of those fractions: the largest (>100-micron fraction), which includes the vast majority of *Microcystis* colonies from western Lake Erie (Chaffin et al., 2011) and attached microbes (hereafter, the "colony" fraction), and the smallest (0.8-20 micron fraction), which we expect included most fungal spores (hereafter, "zoospore" fraction), although fungi also infecting or attached to small (>20 micron) algae could also be included. Material collected on the 100-micron and 20-micron mesh was backwashed and collected and then divided in half by volume, with one half preserved in 95% ethanol and the remaining half preserved in 10% glycerol and stored at -80 °C. After passing through the 100- and 20-micron mesh, the water was then filtered through 142 mm diameter 0.8 micron Isopore polycarbonate filters (Millipore) using a peristaltic pump, after which 0.8 micron filters were cut into quarters. We filtered 10 L of water from each sample when possible; however, smaller volumes (minimum sample volume = 1 L) were used when the water clogged the filter before a

full 10 L sample was filtered (i.e., the water contained enough particulate matter in the 0.8-20 micron range the filter pores were clogged to the point that flow-through slowed greatly). The filter quarters were then immediately transferred to cryovials and frozen at -80 °C; one quarter was frozen as is or in the lysis buffer described below, one quarter was stored in 95% ethanol at -80 °C, and two remaining quarters were stored in 10% glycerol. The filter portions used for DNA extraction in this study were those either frozen as is or preserved in ethanol (see below). All equipment and collection bottles were sanitized with 10% bleach and rinsed with Milli-Q water between samples.

DNA Extraction

For the colony fraction, the ethanol-preserved samples were centrifuged and most ethanol was removed via decanting. The remaining pellet was transferred to a microcentrifuge tube and the remaining ethanol evaporated in a vacuum centrifuge. DNA was extracted following a modified version of the protocol of (Gast et al., 2004). We added 200 µl lysis buffer (100 mM Tris [pH 8.0], 40 mM EDTA, 100 mM NaCl, and 1% sodium dodecyl sulfate, heated to 70 °C) to the samples, and then we added approximately 200 µl of zirconia-silica beads (0.5-mm diameter; BioSpec Products, Inc.). Samples were vortexed 1 min, incubated at 70 °C for 5 min, and then vortexed again for 1 min, followed by another incubation at 70 °C for 5 min and 1 min vortexing. We then added 5 M NaCl to bring the solution to 0.7 M NaCl, followed by addition of 10% hexadecyltrimethylammonium bromide (CTAB) to the sample to a final concentration of 1%. Samples were incubated at 70 °C for 15 min. We then added an equal volume of chloroform, vortexed and centrifuged the sample (12 min at 12.5K rpm), and then removed the aqueous (top) layer to a sterile 1.5-ml microcentrifuge tube. Nucleic acids were precipitated by addition of 0.6 volumes of isopropanol, which were then recovered by centrifugation (10 min at 12.5K rpm). The pellet was washed with 1 mL cold (-20 °C) 70% EtOH, supernatant removed, the pellet dried using a vacuum centrifuge, and the sample was resuspended in 50 µl of sterile molecular grade water.

For the zoospore fraction, we used the filter quarter that was frozen as is if available or else the ethanol-preserved quarters. If frozen as is, the filter quarter was allowed to thaw immediately before the DNA extraction; if preserved in ethanol, the filter quarter was removed from ethanol, placed in a sterile petri dish, and allowed to dry under a laminar flow hood. The filter quarter was

then moved to 2 mL microcentrifuge tube, cut into ~10 pieces, and then DNA was extracted following the same steps as the colony fraction.

PCR

Extracted DNA was then amplified using PCR with two barcoded primer sets: general fungal primers targeting the ITS region (ITS-1F and ITS4, Gardes and Bruns, 1993) and Cryptomycota-preferential primers targeting the 18S rRNA gene region (CRYPTO2-2F and AU4v2, Lazarus and James, 2015). PCR reactions were prepared on ice according to the following recipe for a total reaction volume of 12.5 µl: 2.94 µl molecular grade water, 1.25 µl 10X ExTaq buffer, 1 µl $MgCl_2$ (25 mM), 1 µl dNTPs (2.5 mM each), 0.625 µl forward primer (10 µM concentration), 0.625 µl reverse primer (10 µM concentration), 0.0625 µl ExTaq (TaKaRa; 5U/µl), and 5 µl of genomic DNA (diluted to ≈ 0.5 ng/µl). For ITS, the PCR temperature profile was as follows: 94 °C for 3 min followed by 25 cycles of 94 °C for 45 s, 53 °C for 30 s, and 72 °C for 2 min, followed by a final extension at 72 °C for 7 min. For 18S, the PCR temperature profile was as follows: 94 °C for 3 min followed by 35 cycles of 94 °C for 1 min, 58 °C for 30 s, and 72 °C for 1 min, followed by a final extension at 72 °C for 7 min.

PCR products that showed successful amplification (visible bands in the appropriate size range after gel electrophoresis) using each primer pair (ITS: 60 samples from WLE and 14 samples from SB; Crypto: 40 samples from WLE and 11 samples from SB) were purified using a Minelute PCR Purification Kit (Qiagen). The DNA concentration of each sample was measured using a Qubit fluorometer. For each sequencing run, multiple samples were diluted to equivalent concentrations and then pooled so that an equal amount of DNA was included from each sample on a given sequencing run. Samples were submitted to the University of Michigan DNA Sequencing Core for sequencing using a PacBIO RS II sequencer. Sequencing runs included both samples from both this study and similar samples from a study on inland lakes. 110 samples (73 from this study) were included on 5 SMRT chips (22 samples pooled for each SMRT chip) for ITS and 69 samples (52 from this study) were included on 7 SMRT chips (9-10 samples pooled for each SMRT chip) for Cryptomycota-preferential primers.

Sequence Processing and OTU Assignment

Circular consensus sequences (CCS) with a minimum of 5 passes were extracted from the data generated from each SMRT chip (132,968 sequences for ITS; 227,488 sequences for 18S). Sequences were processed using mothur v. v.1.44.1 (Schloss et al., 2009) and Qiime (Caporaso et al., 2010). Sequences were demultiplexed and trimmed in mothur using the trim.seqs command with the following parameters: qaverage = 41, maxambig = 2, maxhomp = 20, bdiffs = 1, pdiffs = 3, minlength = 300. Chimeras were detected and removed using the uchime algorithm implemented in mothur. OTUs were assigned in Qiime using a 3% cutoff and classified via comparison to the UNITE (v. 8.2) database for ITS (Koljalg et al., 2013) and a modified version of the SILVA (v. 138) reference database (Quast et al., 2013) for 18S data. 78.6% of the sequences in the ITS data set and 20.9% of the sequences in the 18S data set were not classified as fungal (primarily protists, animals, or unidentified eukaryotes). Because our focus is on the fungal community, nonfungal sequences were removed prior to further analysis. OTUs that occurred fewer that 4 times were also removed as additional quality control to further reduce the likelihood of a lower quality sequence or chimera being included in the final analysis. Representative sequences from the 20 most common fungal OTUS in the 18S data set and additional reference sequences were used to construct a phylogenetic tree to provide additional information on OTU identify (Electronic Supplementary Material (ESM) Appendix S1). Finally, samples with fewer than 100 fungal sequences were removed (the smallest samples remaining included 113 sequences for ITS, 260 sequences for 18S).

Community Analyses

Community analyses were performed in mothur and R version 3.6.3 (R Core Team, 2020). In mothur, we calculated a rarefied number of observed OTUs and inverse Simpson's diversity index using a subsample of 113 sequences from each sample for the ITS data or 260 sequences for the 18S data. In R, we used ANOVA to analyze differences in fungal alpha diversity (observed number of OTUs and Inverse Simpson's index) among lakes, sites, dates, and fractions. We performed two sets of ANOVAs for each data set (ITS and 18S). First, to compare between lakes, we focused on data from samples from western Lake Erie and Saginaw Bay that were approximately contemporaneous (4 dates per lake, samples from different lakes collected approximately within 1 week of each other). Second, to compare among sites within Lake Erie (3 WE sites), we included available data from all dates; we focus on western Lake Erie for this

second analysis because of the larger amount of data available for western Lake Erie (3 sites, 11 dates) compared to Saginaw Bay (2 sites, 4 dates). Due to a limited number of samples with sufficient data (see results), analysis of the ITS data did not include a test for the 3-way lake x fraction x date interaction in the cross-lake analysis or tests for any interactions in the cross-site analysis of alpha diversity. Spearman rank correlation tests were used to test the relationships between measures of alpha diversity or the relative abundances of particular taxa (e.g., % of sequences belonging to Chytridiomycota) and measures of primary producer and cyanobacterial biomass and toxicity (chlorophyll a, phycocyanin, and particulate microcystin concentrations) that relate to the magnitude of the bloom at a given site on a given date. Data for chlorophyll a, phycocyanin, and particulate microcystin concentrations were measured from depth-integrated water samples collected simultaneously with our samples by researchers from NOAA-GLERL and CIGLR (Burtner et al., 2019).

To examine differences in community composition among our samples, we generated a distance matrix by calculating Yue and Clayton's theta value (Yue and Clayton, 2005), which considers the proportions of both shared and non-shared taxa in each sample and is commonly used in analysis of microbial community data (e.g., Kozich et al., 2013), using the dist.shared command in mothur. The distance matrix was then used in the adonis function in the R vegan package (Oksanen et al., 2019) to test if community composition differed between lakes, sites, dates, and fractions, and to test for associations between composition and metrics of bloom magnitude (chlorophyll a, phycocyanin, and particulate microcystin concentrations). As with the ANOVA analysis above, we again performed two sets of analyses for each data set (across lakes and across sites within Lake Erie). Non-metric multidimensional scaling (NMDS) analysis implemented in mothur was also used to visualize patterns in community composition and correspondence with environmental factors (chlorophyll a, phycocyanin, microcystin). Finally, we also tested for correlations between the most common 10 OTUs in each data set and metrics of bloom magnitude using a Spearman correlation test.

Results

ITS

After processing for quality and removing non-fungal sequences, the ITS data included 13,954 sequences from 44 samples that included at least 113 sequences per sample. These

samples included 35 samples from western Lake Erie (10 colony fraction, 25 zoospore fraction) and 9 samples from Saginaw Bay (2 colony fraction, 7 zoospore fraction). The study duration coincided with blooms in both Great Lakes; weekly measurements of pigment concentrations by NOAA-GLERL and CIGLR in western Lake Erie reflect two major bloom events that occurred with peaks in early August and late September (Fig. 1, Berry et al., 2017), while pigment and toxin concentrations indicate that the bloom in Lake Huron peaked in late July (NOAA-GLERL Data).

The most common fungal taxa (phylum level, making up >1% of sequences) across all samples were Chytridiomycota (54.8%), unidentified fungi (34.2%), Basidiomycota (4.7%), Ascomycota (3.2%), Blastocladiomycota (1.9%), and Cryptomycota (=Rozellomycota, 1.2%) (ESM Fig. S1). After removing OTUs that occurred fewer than 4 times, there were 240 total OTUs in the ITS data. The identities and overall relative abundance (percentage of sequences) of the most common OTUs for both the ITS and 18S markers are listed in ESM Table S1. The ANOVA results indicate that the number of OTUs observed and the inverse Simpson's index did not differ between sites or lakes (Table 1). In the cross-lake analysis, both the number of OTUs observed and the inverse Simpson's index differed between size fractions and across dates, while no interactions were significant. In the cross-site analysis, fractions differed in the number of OTUs observed, but no other effects were significant. Both metrics were significantly higher in the zoospore fraction than colony fraction (Table 1; Fig. 2). The number of observed OTUs was positively correlated with chlorophyll a concentration but not phycocyanin or microcystin concentrations; the inverse Simpson index was not correlated with any of the three (Table 2). For the zoospore fraction, the percentage of sequences in samples belonging to Chytridiomycota was positively correlated with concentrations of chlorophyll a but not phycocyanin or microcystin, while the percent of sequences corresponding to Cryptomycota was positively correlated with microcystin but not chlorophyll a or phycocyanin (Table 3; Fig. 3); for the colony fraction, the percentages of those taxa were not correlated with chlorophyll a, phycocyanin, or microcystin.

Analysis of community composition using adonis revealed significant differences between western Lake Erie and Saginaw Bay, between sites in Lake Erie, between fractions, and among samples collected on different dates (Table 4; ESM Fig. S2a; Fig. 4). The lake x fraction, lake x date, and lake x fraction interactions for the cross-lake analysis and the site x date interaction for the cross-site analysis were also significant. For the NMDS analysis, we included 4 dimensions

to allow for an acceptable stress level (stress level = 0.196, $R^2 = 0.51$). Composition was significantly associated with chlorophyll a (F = 1.61, P = 0.022), phycocyanin (F = 2.12, P = 0.002), and microcystin concentrations (F = 2.05, P = 0.004; Fig. 4).

Analysis examining correlations between the percentage of sequences belonging to the ten most common OTUs with bloom metrics (chlorophyll a, phycocyanin, and microcystin) indicated that three OTUs (ITS OTUs 1, 3, and 4; ESM Table S1) were positively correlated with at least one bloom metric, and one OTU (ITS OTU 10) was negatively correlated with bloom metrics.

18S

After processing for quality and removing non-fungal samples, the 18S data generated using Cryptomycota-preferential primers included 75,778 sequences from 46 samples (western Lake Erie: 36; Saginaw Bay: 10; at least 260 sequences per sample after excluding one sample with only 2 sequences). The most abundant group (phylum level) across taxa was Chytridiomycota (45.4%), followed by Aphelidiomycota (39.1%), and Cryptomycota (13.8%) (ESM Fig. S3). Members of Neocallimastigomycota (0.7%), Ascomycota (0.6%), and Basidiomycota (0.5%) were also present in samples.

After removing OTUs that occurred fewer than 4 times, the samples included 948 OTUs. The number of OTUs observed differed among lakes, sites, fractions, and dates (Table 1; Fig. 5). A significant lake x date interaction in the cross-lake analysis and site x date interaction in the cross-site analysis suggest that changes in OTU richness over time varied between the lakes and among sites in Lake Erie. A significant fraction x date interaction in the cross-site analysis (marginally not significant in the cross-lake analysis) indicates differences among fractions in temporal variation in OTU richness. Fractions also differed in the inverse Simpson's index and there were significant lake x fraction and lake x date interactions in the cross-lake analysis, while all other effects and interactions were not significant. Neither the number of OTUs observed nor the inverse Simpson's index correlated with chlorophyll a, phycocyanin, or microcystin concentrations (Table 2). The percentages of sequences belonging to Chytridiomycota, Cryptomycota, or Aphelidiomycota also were not correlated with chlorophyll a, phycocyanin, or microcystin (Table 3).

Analysis of community composition with adonis indicated significant main effects of lake, fraction, and date (ESM Fig. S2; S4) but not site (Table 4). The cross-lake comparison indicated all interactions were significant, while the cross-site analysis did not indicate any significant interactions (Table 4). For the NMDS analysis, we included 4 dimensions to allow for an acceptable stress level (stress level = 0.17, $R^2 = 0.74$). Composition was not significantly associated with chlorophyll a, phycocyanin, or microcystin concentrations ($P > 0.1$).

Analysis examining correlations between the percentage of sequences belonging to the ten most common OTUs with bloom metrics (chlorophyll a, phycocyanin, and microcystin) indicated that two OTUs (18S OTUs 2 and 10; ESM Table S1) were positively correlated with at least one bloom metric. The phylogenetic analysis of these OTUs showed that the top two most common OTUs were members of the Aphelidiomycota, while other common OTUs grouped in orders of Chytridiomycota that are known to contain parasites of algae, such as the Rhizophydiales, Gromochytriales, Mesochytriales, and Lobulomycetales (ESM Figure S5).

Discussion

Our results indicate that a diverse community of fungi occurs within western Lake Erie and Saginaw Bay and, consistent with our hypothesis, revealed that major shifts occur in the fungal community associated with HABs in western Lake Erie. In particular, analysis of the ITS data indicated that the relative abundance of the Chytridiomycota and Cryptomycota increased in association with measures of bloom magnitude (Chytridiomycota and Cryptomycota were positively correlated with chlorophyll a and microcystin concentrations, respectively). We also saw evidence for some significant associations between both fungal alpha diversity and community composition and measures of bloom magnitude. A caveat is that our data are observational in nature, which limits our ability to directly assess the functional role of observed taxa or rule out that observed shifts may have occurred in response to other environmental factors that changed synchronously with the blooms. Nevertheless, these results are suggestive that the fungal community may be directly interacting with the cyanobacteria causing harmful blooms and may influence bloom timing, magnitude, and duration.

Our findings add to the small number of studies that have examined fungi within the Great Lakes (e.g., Kiziewicz and Nalepa, 2008; McKindles et al., 2021; Wahl et al., 2018) and the association of fungal communities with HABs (Chen et al., 2010; Zhang et al., 2018). We found

a high level of fungal diversity in the lakes, including many novel sequences with loose affiliation with well-characterized diversity, the so called "dark matter fungi" (Grossart et al., 2016). Others are classifiable to phylum and below and could be hypothesized to influence key ecological processes. In particular, the fungal communities were dominated by the Chytridiomycota, which are the major fungal pathogens of algae in aquatic ecosystems (Grossart et al., 2019; Rasconi et al., 2012). For example, the large, but polyphyletic, genus *Rhizophydium* was observed in our samples and is known to include parasites of *Microcystis* (Rasconi et al., 2009), although corroborating visual evidence of infection via microscopy will necessary before making a definitive claim that the *Rhizophydium* in our samples are parasitic on *Microcystis*. Other chytrids have been described from *Planktothrix* and other cyanobacteria (McKindles et al., 2020; Rohrlack et al., 2015). Our phylogeny shows that 5 of the top 20 18S OTUs group within the Rhizophydiales (ESM Fig. S5). In addition to chytrids, we also saw representation from the more recently discovered fungal lineages, such as the Cryptomycota and Aphelidiomycota, which are putative obligate intracellular parasites known primarily from other environmental metabarcoding studies (Karpov et al., 2014; Lazarus and James, 2015). Cryptomycota are mostly known as parasites of heterotrophs, such as chytrids (Gleason et al., 2012); and aphelids are documented only as parasites of algae, particularly green algae and diatoms (Karpov et al., 2014). No aphelids are recorded from cyanobacteria, but aphelids are known as parasites of microalgae (Karpov et al., 2014), and may yet turn out to be parasitic on *Microcystis* and be the source of many of the uncharacterized fungi in our study. Alternatively, there are many other algal taxa that thrive under the same conditions and co-occur with *Microcystis* that could be suitable hosts for the aphelids, and it is known that the blooms may coincide with a diversity of eukaryotic algae (Binding et al., 2019).

In addition to potential interactions with cyanobacteria, some observed fungal taxa associated with HABs may be parasitizing other phytoplankton (e.g., diatoms and green algae), zooplankton, or other fungi (Gleason 2008), which may also have important ecological consequences for the blooms. For example, impacts of fungal parasites on other phytoplankton may facilitate population growth of dominant cyanobacterial species, such as *M. aeruginosa*, by limiting growth of potential competitors. Fungal parasites may also influence the lake food web both through direct effects on algae but also potentially by influencing food availability and

quality for zooplankton, as fungi can facilitate grazing on cyanobacteria, and fungal zoospores can serve as a food item (Frenken et al., 2018; Haraldsson et al., 2018).

Besides impacts of fungal parasitism on algal populations and food web dynamics, the diverse fungi observed also likely influence other important ecological processes in the lake. Chytrids, members of the Dikarya, and other observed taxa include important consumers and decomposers in aquatic systems (Grossart et al., 2019) and play a critical role in the cycling of key elements (Danger et al., 2016; Gulis et al., 2006), such as carbon and nitrogen, which are critical for the functioning of freshwater systems. Observed shifts in community composition thus likely influence key rates of decomposition and nutrient cycling. The role of fungi as decomposers (Gulis et al., 2006) also may be particularly important in processing the large amount of biomass produced during large blooms. In addition, some detected taxa may have entered the water from benthic sediment resuspension or from soils in the Lake Erie watershed due to presence in runoff. While some of these taxa may be present in the lakes in an inactive state, other such taxa may have important functional consequences. For example, fungi originating from the surrounding watershed can function as ice-nucleating particles that influence the water cycle (e.g., Knackstedt et al., 2018) or actively infect aquatic organisms (e.g., Garrison et al., 2003). The variety of functional roles of fungi in the lakes and range of environmental factors affecting those roles may explain why some metrics were not significantly associated with the bloom (e.g., alpha diversity and community composition from the 18S data). Differences in sample volume filtered may also have influenced the taxa observed in different samples, although biases should have been minimized by equalizing DNA concentrations among samples when they were pooled, rarefaction prior to community analyses, and the focus of our analysis on relative (rather than absolute) abundance of different taxa; differences in sample volume are thus unlikely to have influenced observed patterns. In any case, caution must be applied to infer functional importance from our sequencing data; future work examining (e.g., using RNA to assess gene expression) may offer deeper insights into the functional importance of different fungi in these lakes.

Besides associations with bloom magnitude, we also detected variation in the fungal community depending on the fraction of the water column examined, the date of sample collection, and the lake and site of sample origin. For some metrics, we detected different patterns depending on whether we examined data for the ITS or 18S marker; such differences

may have occurred due to differences in which samples successfully amplified fungal DNA and thus were included in our analysis and differences in the taxa targeted by each primer pair (i.e., general fungal for ITS, zoosporic fungi-targeted for 18S). Differences between the colony and zoospore fractions suggest that the microbial communities associated with larger particulate matter, which included most *M. aeruginosa* colonies, differ from free-living fungi. Significant effects of date, site, and lake likely arose due to variation over time and space in environmental factors (e.g., temperature, phytoplankton composition, water quality) that likely contributed to differences in the fungal community. Significant effects of date indicate temporal shifts in community composition occurred, although data from additional years will be necessary to determine whether observed shifts are consistent with seasonal shifts that happen each year or less predictable. Differences between lakes and significant interactions between the effect of lake and other factors (date and fraction) suggest distinct fungal communities occurred in western Lake Erie and Saginaw Bay; the lake x date interactions suggest that dynamics of the fungal communities also differed between lakes, which could relate to differences between the lakes in bloom dynamics (e.g., bloom magnitude has recently increased in Lake Erie but has not changed substantially in Saginaw Bay; Sayers et al., 2016; Wynn et al., 2021). Within Lake Erie, site also had some influence, which was expected due to the close proximity of one site (WE2) to the Maumee River inlet, where bloom magnitude tends to be greatest, in contrast to the offshore site (WE4), which tends to be less directly affected by blooms.

Our results motivate next steps to explore the associations between fungi and HABs. Most importantly, the individual fungal taxa of most importance should be microscopically inspected, using either light microscopy to identify chytrid thalli or mycelial forms or using fluorescent in situ hybridization probes targeting the most abundant and phylogenetically interesting taxa. As our analyses focused on a single year of observational data, it will be important in future work to examine data over multiple years to assess potential contrasts in fungal communities under different conditions. Further, experimental work may allow for more understanding of the potential factors that mediate interactions between fungi and cyanobacteria (e.g., nutrients, temperature). Experiments may also offer additional insights into how variation in the amount of algal biomass influences the fungal community. For example, a possible future study could involve comparing changes in the fungal community over time in laboratory mesocosms either including or excluding the largest fraction that contains large *Microcystis* colonies. Finally,

exploring the functional consequences of changes in the fungal community will be important for assessing potential impacts on bloom magnitude or other key dynamics in the lake (e.g., other phytoplankton populations, zooplankton grazing, nutrient cycling) and freshwater ecosystems more generally.

Conclusions

Our results reveal a diverse community of fungi occurs in western Lake Erie and Saginaw Bay during *Microcystis* blooms, including understudied fungal lineages that are known to parasitize algae, such as chytrids and aphelids. Further, the observed fungal communities varied over time and space in association with the HAB, consistent with our hypothesis that fungal communities shift due to the role of some taxa as parasites or as decomposers responding to impacts of changing available organic matter. The findings are useful to identify potentially important fungal pathogens of HAB-forming cyanobacteria and suggest that fungi may have potentially important interactions with HAB dynamics in the Great Lakes and other systems. These findings thus motivate future work to better understand fungal-HAB dynamics and ecosystem scale impacts of fungi in large lakes.

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Table 1: ANOVA results for the number of observed OTUs (sobs) and inverse Simpson diversity index (invSimpson) for ITS and 18S data, comparing across Lakes (Erie or Huron) or sites (WLE2, WLE4, WLE12).

	Across Lakes											
	ITS						**18S**					
	sobs			invSimpson			sobs			invSimpson		
	F	df	P	F	df	P	F	df	P	F	df	P
Lake	0.98	1	0.345	1.44	1	0.256	13.31	1	**0.003**	1.37	1	0.262
Fraction	4.89	1	**0.0491**	13.99	1	**0.003**	58.05	1	**>0.001**	14.94	1	**0.002**
Date	4.39	3	**0.0292**	7.13	3	**0.006**	6.33	3	**0.007**	2.35	3	0.120
Lake x Fraction	0.44	1	0.519	3.97	1	0.072	3.77	1	0.074	6.17	1	**0.027**
Lake x Date	1.01	3	0.425	0.832	3	0.503	3.86	3	**0.036**	7.00	3	**0.005**
Fraction x Date	0.81	2	0.471	1.849	2	0.203	3.18	3	0.060	0.70	3	0.567
Lake x Fraction x Date	NA	NA	NA	NA	NA	NA	4.14	1	0.063	4.35	1	0.057
	Across Sites (Erie Only)											
	ITS						**18S**					
	sobs			invSimpson			sobs			invSimpson		
	F	df	P	F	df	P	F	df	P	F	df	P
Site	0.82	2	0.454	1.52	2	0.241	18.34	2	**0.010**	2.03	2	0.246
Fraction	8.66	1	**0.008**	2.76	1	0.111	217.55	1	**>0.001**	35.09	1	**0.004**
Date	1.60	10	0.176	1.43	10	0.234	6.92	8	**0.040**	2.38	8	0.210
Site x Fraction	NA	NA	NA	NA	NA	NA	0.93	2	0.465	2.379	2	0.209
Site x Date	NA	NA	NA	NA	NA	NA	8.26	13	**0.028**	2.261	13	0.224

Fraction x Date	NA	NA	NA	NA	NA	NA	9.78	5	**0.023137**	3.30	5	0.135

Table 2: Spearman rank correlation for the number of observed OTUs (sobs) and inverse Simpson diversity index (invSimpson) vs. chlorophyll a, phycocyanin, or microcystin concentrations in Lake Erie sites for ITS and 18S data.

	ITS				**18S**			
	Zoospore Fraction							
	sobs		invSimpson		sobs		invSimpson	
	rho	P	rho	P	rho	P	rho	P
Chlorophyll a	0.40	**0.046**	0.39	0.057	0.079	0.771	0.097	0.721
Phycocyanin	0.26	0.218	0.12	0.581	0.18	0.498	0.17	0.519
Microcystin	0.069	0.743	-0.030	0.872	0.52	**0.037**	0.32	0.227
	Colony Fraction							
	sobs		invSimpson		sobs		invSimpson	
	rho	P	rho	P	rho	P	rho	P
Chlorophyll a	-0.018	0.973	-0.43	0.218	0.15	0.509	0.05	0.846
Phycocyanin	-0.30	0.407	-0.48	0.166	0.31	0.227	0.27	0.294
Microcystin	-0.44	0.206	-0.59	0.073	0.35	0.173	0.35	0.168

Table 3: Results of Spearman correlation tests between percentages of different fungal phyla in samples and concentrations chlorophyll a, phycocyanin, and particulate microcystin.

	ITS				**18S**					
	Zoospore Fraction									
	% Chytridiomycota		% Cryptomycota		% Chytridiomycota		% Cryptomycota		% Aphelidiomycota	
	rho	P	rho	P	rho	P	rho	P	rho	P
Chlorophyll a	0.54	**0.006**	0.35	0.085	0.0059	0.987	0.320	0.908	0.088	0.746
Phycocyanin	0.39	0.055	0.32	0.121	-0.094	0.730	0.14	0.609	0.000	1.000
Microcystin	0.15	0.466	0.54	**0.006**	0.13	0.630	0.23	0.392	-0.24	0.370
	Colony Fraction									
	% Chytridiomycota		% Cryptomycota		% Chytridiomycota		% Cryptomycota		% Aphelidiomycota	
	rho	P	rho	P	rho	P	rho	P	rho	P
Chlorophyll a	-0.26	0.470	-0.043	0.906	0.14	0.579	0.290	0.263	-0.220	0.388
Phycocyanin	0.079	0.838	-0.37	0.290	0.029	0.913	0.093	0.722	-0.076	0.773
Microcystin	0.27	0.455	-0.520	0.123	0.11	0.669	0.085	0.746	-0.11	0.687

Table 4: Results of adonis analysis of community composition using ITS or 18S data comparing between western Lake Erie and Saginaw Bay (across Lakes) or across sites with western Lake Erie.

	Across Lakes					
	ITS			18S		
	F	df	P	F	df	P
Lake	7.28	1	**0.001**	7.16	1	**0.001**
Fraction	5.02	1	**0.001**	5.06	1	**0.001**
Date	4.64	3	**0.001**	3.37	3	**0.001**
Lake x Fraction	4.09	1	**0.001**	2.98	1	**0.004**
Lake x Date	3.23	3	**0.001**	2.23	3	**0.002**
Fraction x Date	1.85	3	**0.007**	1.80	3	**0.007**
Lake x Size x Week	NA	NA	NA	2.03	1	**0.019**
	Across Sites (Erie Only)					
	ITS			18S		
	F	df	P	F	df	P
Site	1.54	2	**0.015**	1.23	2	0.210
Fraction	3.56	1	**0.001**	4.98	1	**0.001**
Date	2.97	10	**0.001**	2.44	8	**0.001**
Site x Fraction	0.77	2	0.900	1.29	2	0.175
Site x Date	1.72	1	**0.017**	1.73	1	0.072
Fraction x Date	1.14	2	0.217	1.03	2	0.391

Figure Legends

Figure 1: Chlorophyll a, phycocyanin, and particulate microcystin (μg/L) concentrations at the WLE2, WLE4, and WLE12 stations measured by NOAA-GLERL and CIGLR (Burtner et al., 2019).

Figure 2: Mean ± SE (across sites) number of OTUs and Inverse Simpson Diversity index in colony and zoospore fractions for ITS data from western Lake Erie (black) or Saginaw Bay (gray). Triangles indicate dates of peak blooms.

Figure 3: Proportion of sequences in ITS data belonging to Chytridiomycota (A-C) or Cryptomycota (D-F) vs. ln-transformed concentrations of chlorophyll a (A,D), phycocyanin (μg/L) (B,E), or microcystin (μg/L) (C,F) in zoospore fraction. Fitted linear regression lines are indicated for significant relationships to show trends (A and F; note: non-parametric Spearman correlation tests were actually used to tests for statistical significance).

Figure 4: NMDS plots for ITS data from western Lake Erie plotted for 1st two axes for zoospore (left) or colony (right) fractions. Colors indicate corresponding (A,B) date or concentrations of (C,D) chlorophyll a, (E,F) phycocyanin, and (G,H) microcystin for each sample.

Figure 5: Mean ± SE (across sites) number of OTUs and Inverse Simpson Diversity index in colony and zoospore fractions for 18S data (generated with Cryptomycota-preferential primers) from western Lake Erie (black dots) and Saginaw Bay, Lake Huron (gray dots). Triangles indicate dates of peak blooms.

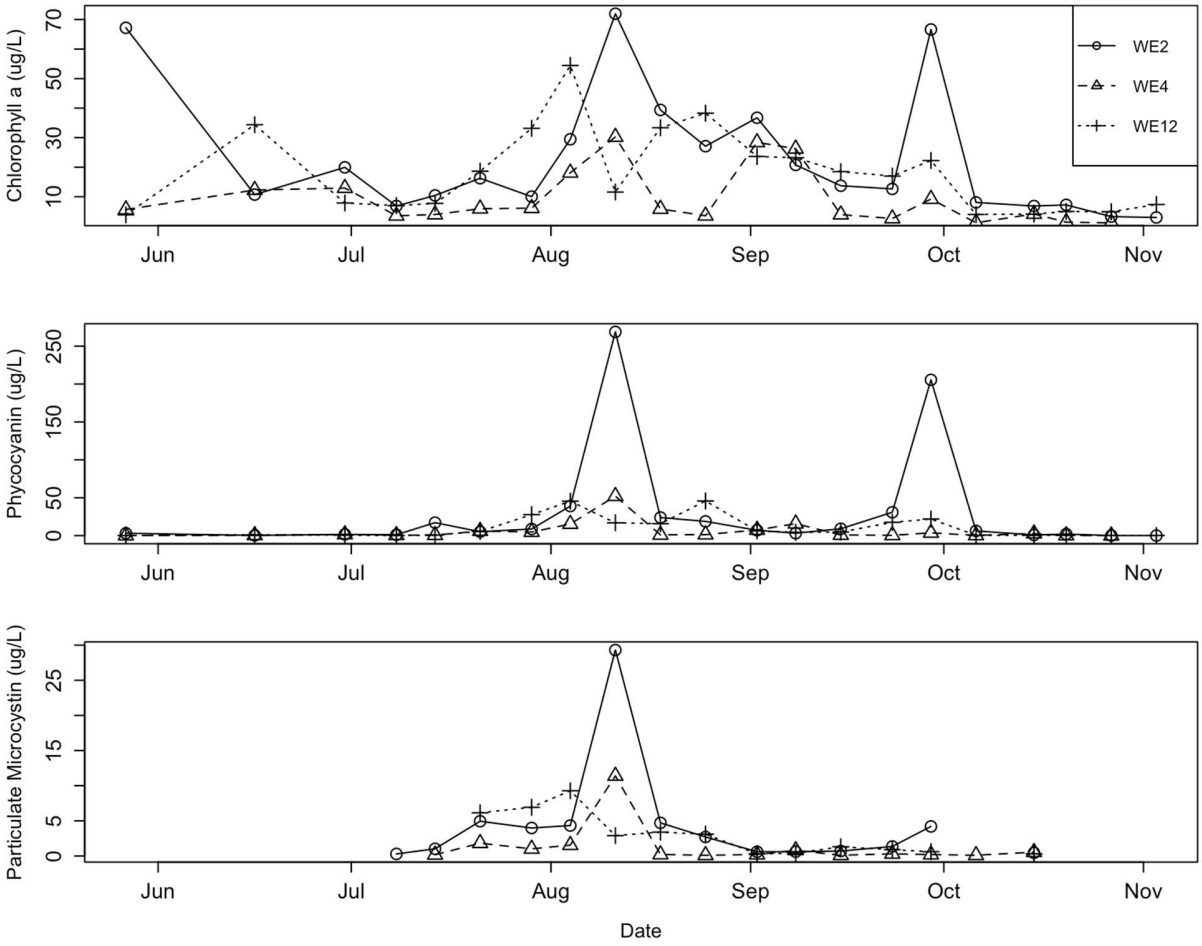
Chlorophyll a (ug/L)
10
30
50
70
WE2
WE4
WE12
Jun
Jul
Aug
Sep
Oct
Nov
Phycocyanin (ug/L)
0
50
150
250
Jun
Jul
Aug
Sep
Oct
Nov
Particulate Microcystin (ug/L)
0
5
15
25
Jun
Jul
Aug
Sep
Oct
Nov
Date

Figure 1

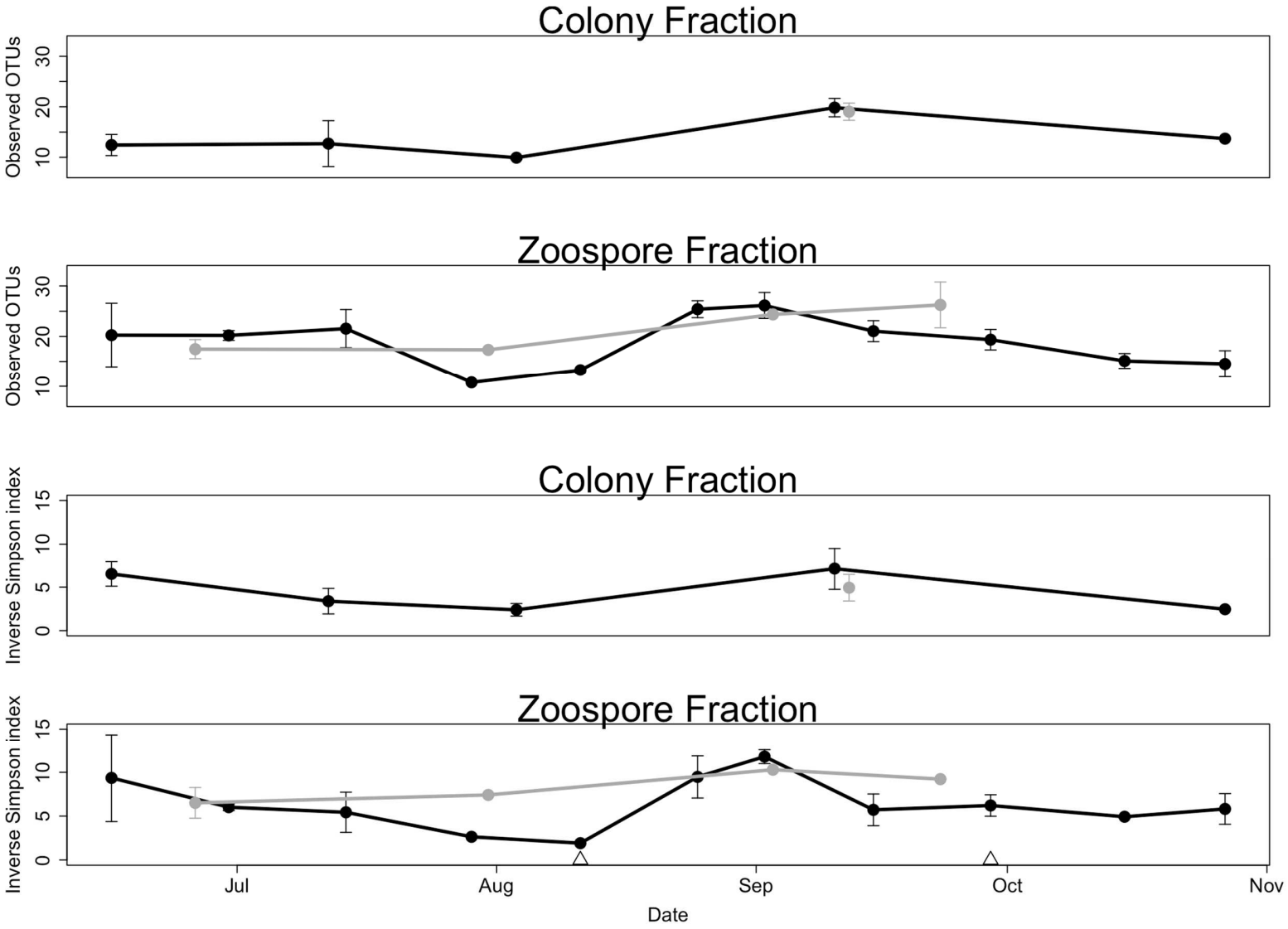
Colony Fraction
Observed OTUs
10
20
30
Zoospore Fraction
Observed OTUs
10
20
30
Colony Fraction
Inverse Simpson index
0
5
10
15
Zoospore Fraction
Inverse Simpson index
0
5
10
15
Jul
Aug
Sep
Oct
Nov
Date

Figure 2

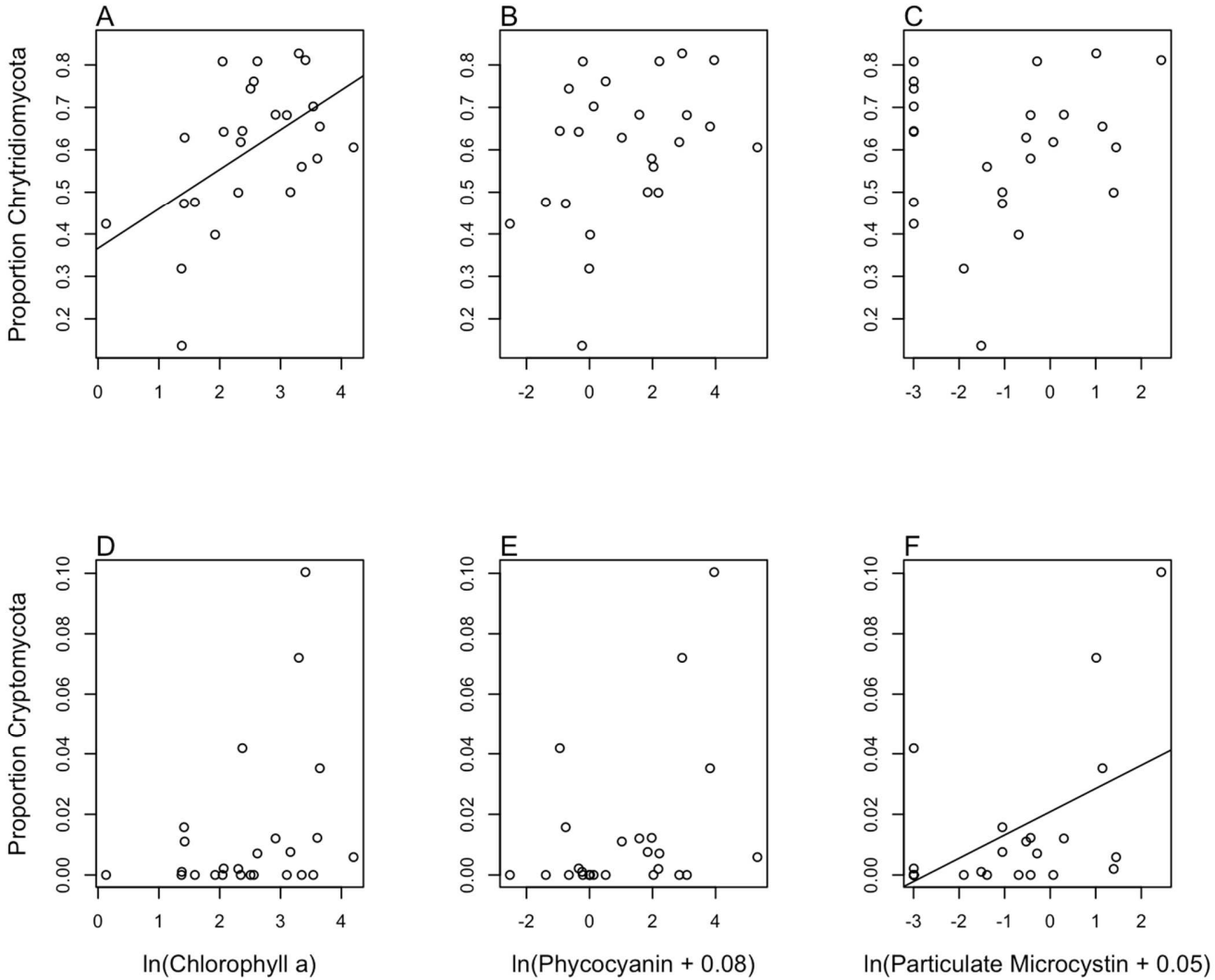
A
B
C
D
E
F
Proportion Chrytridiomycota
Proportion Cryptomycota
ln(Chlorophyll a)
ln(Phycocyanin + 0.08)
ln(Particulate Microcystin + 0.05)

Figure 3

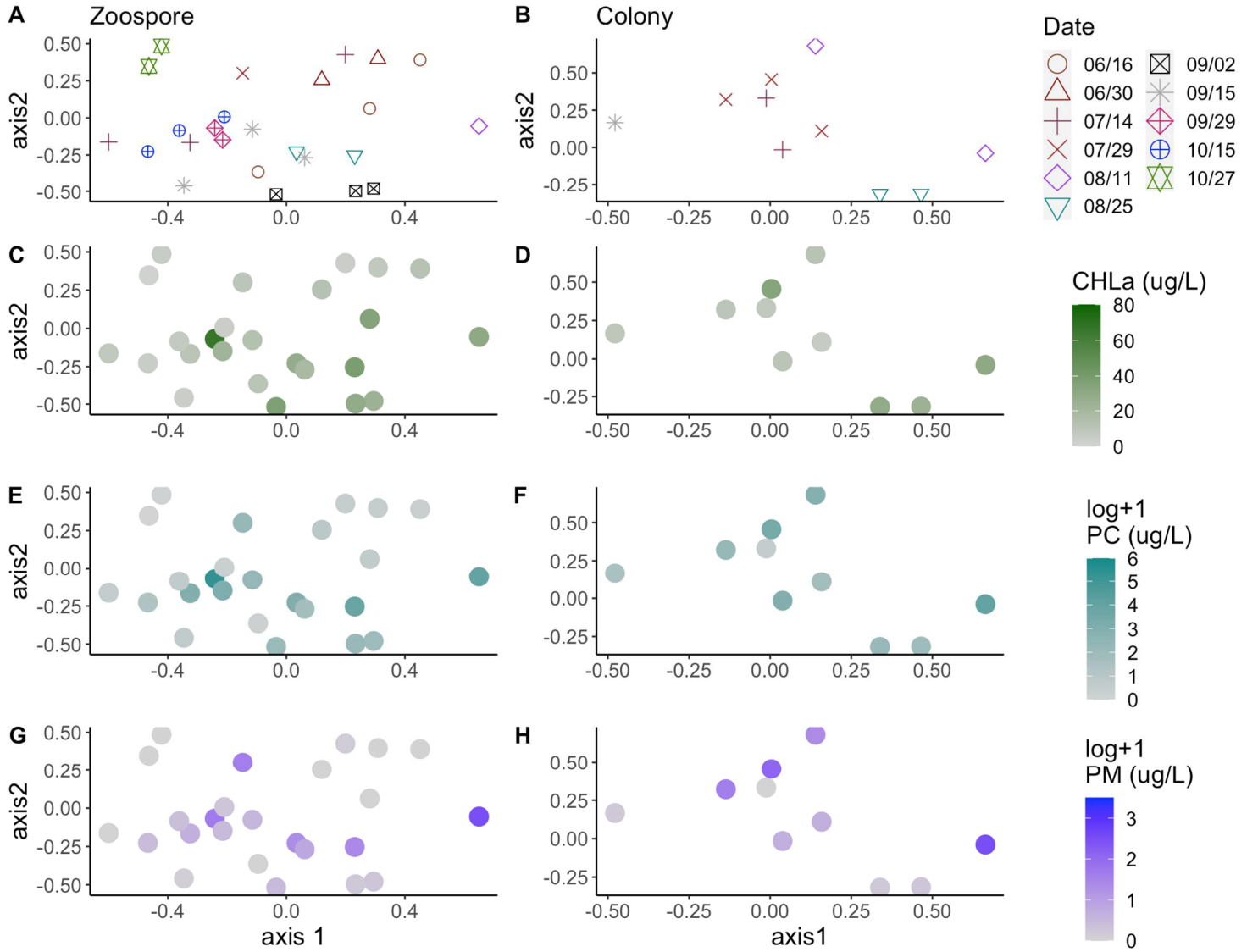
A
Zoospore
B
Colony
C
D
E
F
G
H
axis2
axis 1
axis1
0.50
0.25
0.00
-0.25
-0.50
-0.4
0.0
0.4
-0.50
-0.25
0.00
0.25
0.50
Date
06/16
06/30
07/14
07/29
08/11
08/25
09/02
09/15
09/29
10/15
10/27
CHLa (ug/L)
80
60
40
20
0
log+1
PC (ug/L)
6
5
4
3
2
1
0
log+1
PM (ug/L)
3
2
1
0

Figure 4

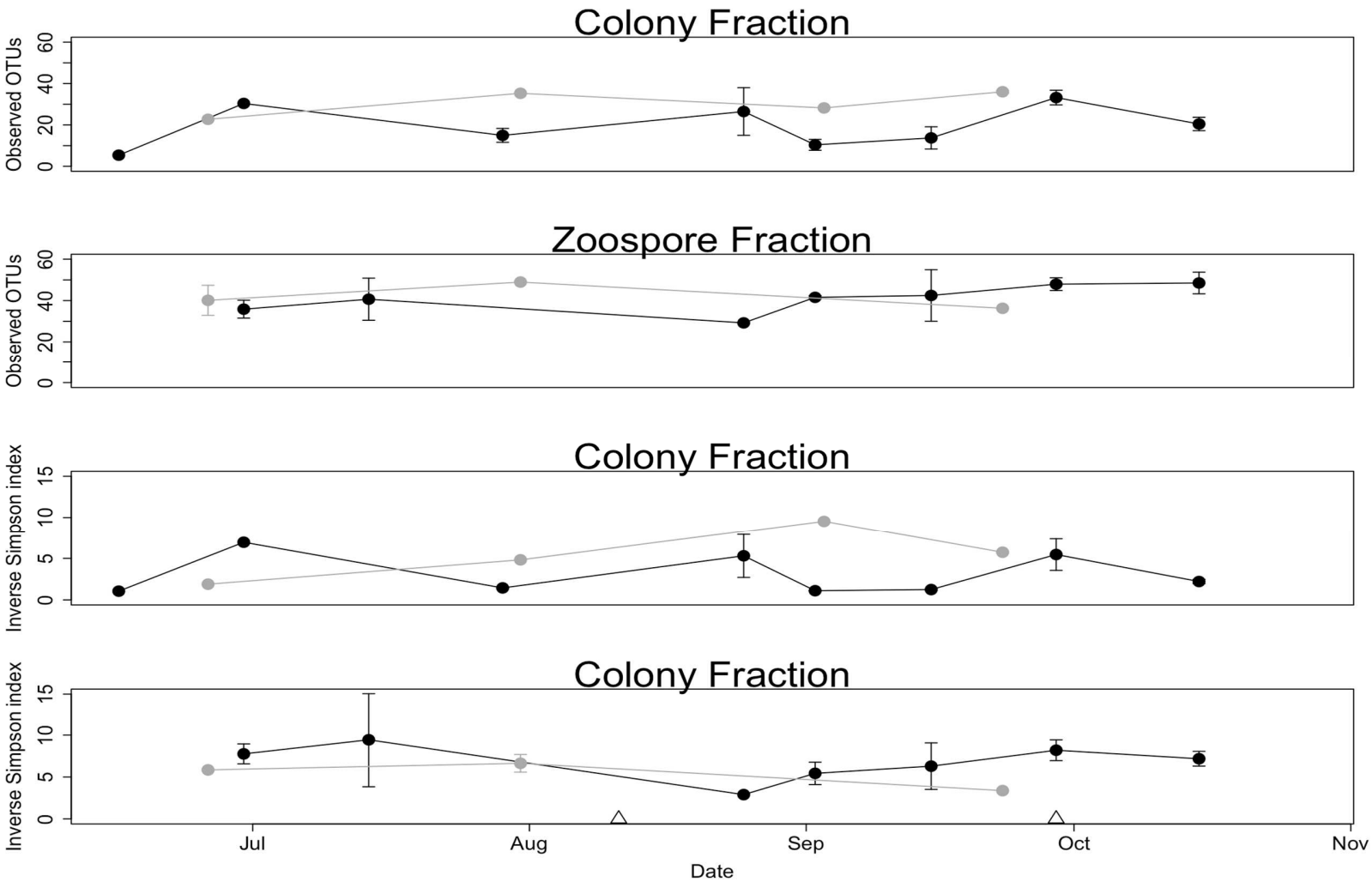
Colony Fraction
Observed OTUs
0
20
40
60
Zoospore Fraction
Observed OTUs
0
20
40
60
Colony Fraction
Inverse Simpson index
0
5
10
15
Colony Fraction
Inverse Simpson index
0
5
10
15
Jul
Aug
Sep
Oct
Nov
Date

Figure 5