Received: 10 February 2022 | Revised: 16 August 2022 | Accepted: 18 August 2022

DOI: 10.1111/mec.16675

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Taxonomic and functional dynamics during chytrid epidemics in an aquatic ecosystem

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Funding information
Agence Nationale de la Recherche, Grant/Award Number: ANR-12-BSV7-009-01; Contrat de Plan État-Région (CPER)

Handling Editor: Suhelen Egan

Abstract

Fungal parasitism is common in plankton communities and plays a crucial role in the ecosystem by balancing nutrient cycling in the food web. Previous studies of aquatic ecosystems revealed that zoosporic chytrid epidemics represent an important driving factor in phytoplankton seasonal successions. In this study, host–parasite dynamics in Lake Pavin (France) were investigated during the spring diatom bloom while following chytrid epidemics using next generation sequencing (NGS). Metabarcoding analyses were applied to study changes in the eukaryotic microbial community throughout diatom bloom–chytrid epidemics. Relative read abundances of metabarcoding data revealed potential "beneficiaries" and "victims" during the studied period. Subsequently, metatranscriptomic analyses on samples before and during the chytrid epidemic unveiled the active part of the community and functional/metabolic dynamics in association with the progress of chytrid infection. Diatom functions involving lipases, transporters, histones, vacuolar systems, the proteasome, proteases and DNA/RNA polymerases were more abundant during the diatom bloom. Chytrid functions related to a parasitic lifestyle including invasion, colonization and stress tolerance were up-regulated during the chytrid epidemic. In addition, functions related to the degradation/metabolism of proteins, lipids and chitin were in higher proportion in the community during the epidemic event. Results of NGS and bioinformatics analyses offered a panorama of dynamic biodiversity and biological functioning of the community.

KEYWORDS
chytrid epidemic, diatom bloom, host–parasite interactions, metabarcoding, metatranscriptomics, mycoloop

1 | INTRODUCTION

Parasites are ubiquitous and represent one of the most described symbiotic interactions in nature (Cavalier-Smith, 1993; Lafferty et al., 2006). They play significant roles in almost all environments by shaping the food-web structure, regulating the host population, facilitating energy transfer/nutrient flows and maintaining genetic polymorphism/diversity (Gsell, de Senerpont Domis, van Donk, & Ibelings, 2013; Kagami et al., 2014; Kagami, von Elert, et al., 2007; Marcogliese & Cone, 1997; Monchy et al., 2012). In aquatic

ecosystems, recent ecological and molecular surveys have shown high occurrence and diverse parasites of eukaryotes belonging to the kingdom Fungi (Lefèvre et al., 2007, 2008; Lefranc et al., 2005; López-García et al., 2001; Monchy et al., 2011). Chytridiomycota (chytrids), an early diverging branch of Eumycetes (Barr, 2001; Rasconi et al., 2012), is among the dominant groups of aquatic parasites. More than 700 species of chytrids have been identified as parasites of phytoplankton, zooplankton, fungi and animals (Gleason et al., 2008; Sparrow, 1960). Most chytrids initiate their life cycle by releasing free-living zoospores that actively search for host cells or substrates to develop and reproduce (Barr, 2001; Sparrow, 1960). For example, once zoospores encyst in their diatom host, chytrids use rhizoid systems to penetrate through the frustule girdle of host cells and extract nutrients for the development of mature sporangia, which, in turn, will release new zoospores into the environment (Beakes et al., 1992; Canter, 1967; Kagami et al., 2014). Most chytrids infect specific diatom hosts (Maier & Peterson, 2014; Rasconi et al., 2012) during the host's optimal development phase (e.g., bloom) (Donk & Ringelberg, 1983; Ibelings et al., 2004; Sen, 1987). Because infection results in death of the host, chytrids not only regulate the host population, but also indirectly maintain genetic polymorphism/diversity (Gsell, de Senerpont Domis, Verhoeven, et al., 2013) and community succession. Moreover, chytrid zoospores, rich in fatty acids (i.e., cholesterol, 24-methylene-cholesterol; Weete et al., 2010), represent an excellent food source for zooplankton (Kagami et al., 2014; Kagami, de Bruin, et al., 2007). As a result, chytrid zoospores represent an alternative trophic pathway as the "mycoloop" (Kagami et al., 2014).

The deep volcanic, oligomesotrophic Lake Pavin (France) is characterized by a small water catchment area, an absence of river inflow and low level of human influence. In this lake, chytrid diversity and seasonal occurrence were confirmed by regular microscopic identification of their sporangia, attached to different classes of phytoplankton (Rasconi et al., 2009, 2012), and molecular methods (Jobard et al., 2012; Lefèvre et al., 2007, 2008; Monchy et al., 2011). These studies revealed chytrid–phytoplankton trophodynamics and allowed three orders of Chytridiomycetes to be distinguished: the Rhizophydiales, the Chytridiales and the Zygophlyctidales. A variety of diatom hosts, from large (e.g., *Asterionella formosa*, *Synedra* spp., *Fragilaria crotonensis*) to small algae (e.g., *Cyclotella* spp., *Chodatella ciliata*, *Ankistrodesmus convolutes*), could be infected with corresponding parasitic *Rhizophidium* species during spring–summer and winter periods (Rasconi et al., 2012). According to previous studies of Lake Pavin, diatoms can account for up to 98% of the total phytoplankton biomass production throughout the spring bloom, followed by chytrid epidemics dominated by *Rhizophidium*, with a prevalence of infection reaching 25%–35% (Rasconi et al., 2009, 2012).

In this study, we investigated a chytrid epidemic during the spring diatom bloom using in situ next generation sequencing (NGS) approaches and data analyses. The aims were: (i) to determine the diversity, succession and active members of the eukaryote community; (ii) to identify down- or up-regulated genes throughout the key step of the chytrid epidemic; and (iii) to unveil potential functions/metabolic pathways affecting the whole eukaryote community. To our knowledge, this study is the first in situ gene-expression exploration of diatom–chytrid-specific host–parasite interactions in a freshwater ecosystem. The community succession with regard to the diatom bloom and chytrid infection was investigated using Illumina sequencing of small subunit ribosomal DNA (SSU rDNA) hypervariable tags (metabarcoding), while the gene expression and active metabolic pathways were revealed by NGS of eukaryote mRNA (metatranscriptomics).

2 | MATERIAL AND METHODS

2.1 | Sampling

Samples were collected from Lake Pavin (45°29′41″N, 002°53′12″), an oligo-mesotrophic deep volcanic mountain lake (Z_{max} = 92 m, altitude 1197 m) located in the French Massif Central region,

TABLE 1 Conditions of sampling points and characteristics of samples

	April 26	May 6	May 13	May 21	May 26
Z Secchi (m)	2	2.8	3.1	2.8	3.6
Depth (m)	6	7	6.5	7	12
Temperature (°C)	3.4	5.5	6.2	6.5	5.1
O_2 (%)	97.4	101.8	108	104.9	83.1
Green algae (µg Chl-*a* l^{-1})	3	3.93	4.05	4.33	0.91
Bluegreen algae/cyanobacteria (µg Chl-*a* l^{-1})	0	0	0	0	0.91
Diatoms/brown algae (µg Chl-*a* l^{-1})	11.83	10.23	5.09	3.56	10.78
Cryptophyta (µg Chl-*a* l^{-1})	0	0	0	0	0
Yellow substances	0	0.19	0.2	0.2	0.93
Total concentration (µg Chl-*a* l^{-1})	14.83	14.16	9.14	7.89	12.6
Prevalence of infection (% of infected host cells)	8	22	30	46	40
Estimated chytrid biovolume (μm^3 per sporangia)	18	30	26	34	29

characterized by a permanently anoxic monimolimnion from 60 m depth downwards. This site offers a unique environment with low human influences, characterized by a small surface area (44 ha), about equal to the drainage basin area (50 ha), with no river inflow. Recurrent spring blooms of diatoms occur in the lake between April and May when diatoms form the bulk of the phytoplankton community. Based on earlier works (Rasconi et al., 2012) and weekly lake monitoring during this period, sampling for the NGS study was initiated on April 26, 2013, when chlorophyll reached 10 µg Chl-*a* l^{-1} (Table 1), then on May 6, May 13, May 21 and May 26. During the sampling period, oxygen profiles and temperature fluctuate from 100% O_2 saturation in the surface water (8–12°C) to 60% at 30 m (4°C) (obtained using a multiparametric probe ProODO; YSI, Yellow Springs). Phytoplankton class determination was performed using the BBE FluoroProbe (bbe Moldaenke). Samples were collected between 6 and 14 m where the highest chlorophyll *a* (Chl *a*) fingerprint spectrum for diatoms/brown algae was recorded. Chytrid infection parameters were evaluated according to a previously published method (Rasconi et al., 2009) with the fluorochrome calcofluor-white (CFW) (Sigma-Aldrich; final concentration 2.5%, v/v) and a Zeiss Axiovert 200M epi-fluorescence microscope (Carl Zeiss) using UV excitation (405 nm).

Triplicates of 60 L Lake Pavin water were first prefiltered through a 150-µm pore-sized nylon mesh (to minimize large metazoans and prevent the outcome result being overwhelmed with metazoan sequences), then filtered through 0.6-µm polycarbonate 147-mm filters (Millipore). Filters were stored at −80°C in RNA*later* (Thermo-Fisher). Three filters were prepared for each sampling date; one was designated for metabarcoding and the other two for metatranscriptomics.

2.2 | Nucleic acid extraction

RNA*later* was removed and filters were then rinsed with phosphate-buffered saline (PBS, 1×). Total genomic DNA was extracted from filters using the PowerWater DNA isolation kit (MO-BIO Laboratories) according to the manufacturer's protocol. DNA concentrations were measured with the Qubit 2.0 Fluorometer (Thermo-Fisher). RNA was later extracted from selected filters corresponding to abundances of diatoms or chytrids (according to microscopy observations and our metabarcoding results). Briefly, filters were incubated with 200 µl acid-washed glass beads (425–600 µm, Sigma-Aldrich) in 200 µl PBS (1×) and treated with a course of liquid nitrogen freeze (2 min), thaw and horizontal vortexing (2000 rpm, 1 min) for three repeats. After the removal of filters, extractions were completed using the RNeasy Plant Mini Kit (Qiagen) according to the manufacturer's protocol. Potential DNA contamination was further removed by two DNase (Sigma-Aldrich) treatments (room temperature, 15 min). RNA quantity was assessed by a Nanodrop (Thermo-Fisher) and RNA quality was assessed via a 2100 Bioanalyzer (Agilent).

2.3 | NGS library preparation

2.3.1 | Library for metabarcoding

The 18S ribosomal RNA gene (rDNA) amplicon library preparation and sequencing were performed at the sequencing facility of Genes Diffusion according to the standard metabarcoding workflow of the facility. The V2–V3 region of eukaryotic 18S rDNA was amplified by using adapter-tagged primers 18S-82F (5′-GAAACTGCGAATGGCTC-3′) (López-García et al., 2003) and Euk-516r (5′-ACCAGACTTGCCCTCC-3′; Amann et al., 1990). 18S amplicon libraries were sequenced with the Illumina MiSeq paired-end system (Illumina). Raw sequence reads were deposited in the NCBI sequence read archive (SRA, BioProject PRJNA789723).

2.3.2 | Library for metatranscriptomics

The cDNA library preparation and sequencing were performed at the Plateforme de Genomique LIGAN-PM (Université Lille, CNRS-UMR8199, Lille, France) following the Illumina TruSeq standard mRNA library preparation workflow (Illumina). The cDNA library was sequenced with the Illumina HiSeq 2500 system (Illumina). Raw sequence reads were deposited in the NCBI SRA (BioProject PRJNA789723).

2.4 | Data analyses: rDNA Sequence processing

All 18S rDNA sequences were processed together using MOTHUR version 1.34.0 (Schloss et al., 2009) following the standard operating procedure including normalization (Kozich et al., 2013). The data set was dereplicated to unique sequences and aligned against the SILVA 108 database (http://www.arb-silva.de). Suspected chimeras were removed by using UCHIME (Edgar et al., 2011). After quality filtering, an average of 100,646 rDNA reads per sample were clustered into operational taxonomical units (OTUs) at 97% similarity threshold (Edgar, 2010), using the average neighbour method in MOTHUR. Single singletons, referring to OTUs that have a single representative sequence in the whole data set, were removed as these are probably erroneous sequencing products (Behnke et al., 2011; Kunin et al., 2010; Reeder & Knight, 2009). After normalization of the entire data set, all remaining (964 OTUs) sequences were searched against the PR2 curated database (Guillou et al., 2012) and SILVA 132 database (Quast et al., 2013) by using BLASTN (Altschul et al., 1990). BLASTN results were carefully examined and manually curated before putative taxonomic affiliation was assigned to each OTU. To categorize the impact of the chytrid epidemic on microbial community members, patterns of relative read abundances for each OTU during the studied period were grouped into “beneficiary,” “victim,” “opportunist” and “neutral/unclassified.” The relative read abundance of each OTU was

calculated as follows: (number of reads for OTU *X* on one sampling date)/(total number of reads for OTU *X* for all five sampling dates) × 100%. Each error bar represented the standard deviation of all relative read-abundances within the same category on one sampling date. Only OTUs that had at least 50 reads in one sample were considered in this pattern analysis and OTUs of metazoa were excluded.

2.5 | Metatranscriptomics analyses

Metatranscriptomics data were analysed according to an established workflow (Haas & Zody, 2010), and consisted of (i) read processing, (ii) reference assembly, (iii) read mapping, (iv) differential expression analysis of read counts and (v) identification of genes/functions. For step (i), all reads were quality checked using FASTQC (Andrews, 2010), followed by adaptor trimming and quality trimming using TRIMMOMATIC (Bolger et al., 2014). To generate a metatranscriptome as the mapping reference in step (ii), processed reads were deduplicated using the "Dedupe" function of BBMAP (Bushnell, 2014), and then de novo assembly was performed with TRINITY (Grabherr et al., 2011). For step (iii), all processed reads from step (i) were aligned against the reference metatranscriptome (from step ii) with BOWTIE 2 (Langmead & Salzberg, 2012). Based on the resulting alignment files, tables with counts for each feature were generated by using the htseq-count script in HTSEQ (Anders et al., 2015). These count tables were then taken in as inputs for the differential expression analysis (step iv) that was carried out with DESEQ2 (Love et al., 2014). According to the results of step (iv), differentially expressed feature sequences were annotated on the protein database using BLASTX (Altschul et al., 1990) in order to infer their functions (step v). BLASTX annotation results were manually curated to categorize the function of genes.

2.6 | Cladogram analysis for chytrid affiliation

A cladogram analysis was performed to provide an overview of taxonomic affiliation and specific subgroup assignments for chytrid OTUs found in this study, along with chytrid reference sequences that were previously found in Lake Pavin and public databases. Sequences of 68 OTUs affiliated with chytrids were aligned using MUSCLE (version 3.8.31) (Edgar, 2004) and all positions containing gaps were eliminated using BIOEDIT version 7.2.5 (Hall, 1999). The cladogram analysis was performed on 465 sites that could be unambiguously aligned, using maximum-likelihood (MCL) methods implemented in MEGA7 (Kumar et al., 2016) based on the Tamura–Nei model (Tamura & Nei, 1993). The initial tree for the heuristic search was obtained by applying the neighbour-joining method to a matrix of pairwise distances estimated using the MCL approach. Bootstrap values (>50%) indicated at each node were calculated from 1000 replicates.

3 | RESULTS

The highest concentrations of diatoms and Chl *a* were recorded at the beginning of the study, which indicated the diatom bloom (April 26, May 6), and then showed a decreasing trend (Table 1). The highest prevalence was observed during the diatom decrease (up to 46%) coinciding with the highest sporangia biovolume (May 21, Table 1). Details of physicochemical environment conditions for the study sites can be found in Table S1.

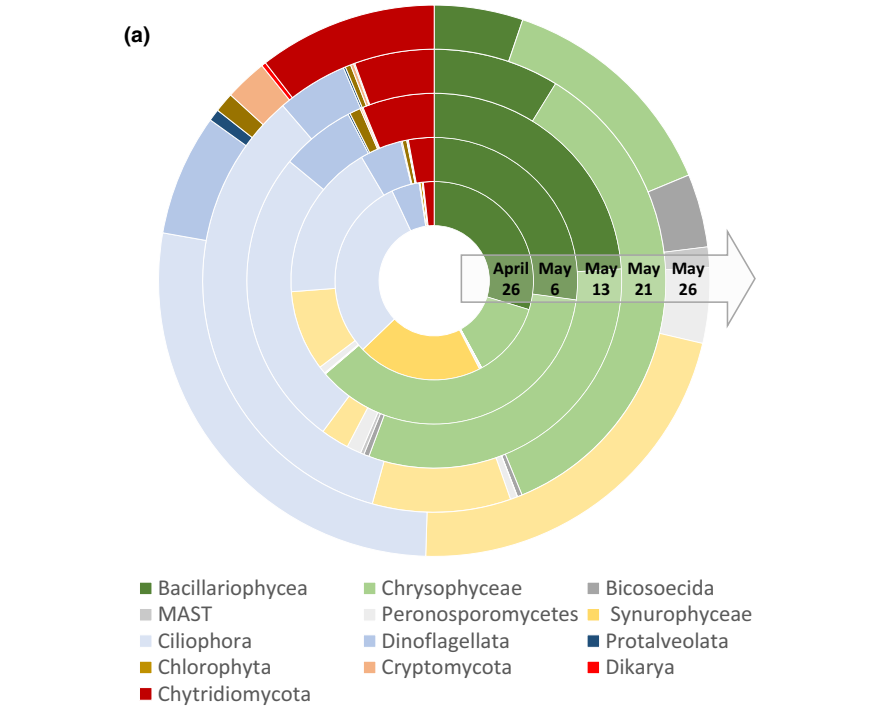
3.1 | Metabarcoding analyses

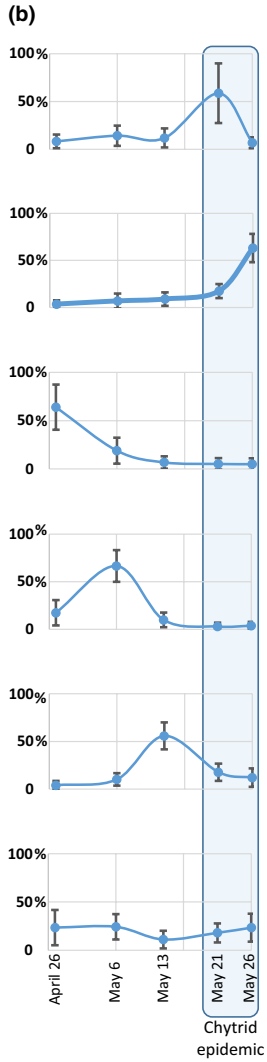
3.1.1 | Impacts of the chytrid epidemic on eukaryotic microbial diversity

A total of 964 OTUs were identified throughout the study using NGS of 18S rDNA amplicons. Lake Pavin microbial eukaryote community sequence reads were dominated by five groups: Ochrophyta (~36 ± 4% of all reads), Chrysophyceae (~24 ± 3%), Ciliophora (~17 ± 4%), Bacillariophyceae (~12 ± 5%) and Chytridiomycota (~4 ± 2%) (Figure 1a). While most taxonomic groups displayed similar relative read abundance throughout the chytrid epidemic, a decrease of Bacillariophyceae and an increase of Chytridiomycota relative read numbers were observed (Figure 1a). During the epidemic, when considering each of the 79 most abundant OTUs (having >50 reads in at least one sample, representing 97.8% of all, nonmetazoan reads), six different patterns of relative read abundance were observed (Figure 1b). These patterns could be classified according to their direct and/or indirect response during the epidemic as: "early/late beneficiary," thrived after the epidemic; "victim," displayed an abrupt decrease of read abundance; "neutral," read abundance was not affected during the epidemic; and "early/late opportunist," read abundance suddenly increased after the diatom bloom and before the peak of the chytrid epidemic (Figure 1b). The highest number of reads affiliated with Chytridiomycota occurred on May 21, 2013, and this date will be considered the peak of the chytrid epidemic (Figure 2). Note that this date also coincided with the highest recorded infection rate and biovolume of sporangia (Table 1).

A total of 19 OTUs that displayed a decrease of relative read abundance were classified as "victims" of the chytrid epidemic. This category is embodied by potential diatom (Bacillariophyceae) hosts of chytrids such as *Aulacoseira granulata*, *Asterionella formosa* and *Stephanodiscus agassizensis*. Other direct or indirect "victims" were affiliated with Oligotrichea (*Rimostrombidium*, *Strombidium* and *Spirostrombidium*), Chrysophyceae (*Paraphysomonas*), Synurophyceae (*Mallomonas*), Dinophyceae (*Biecheleria*, *Symbiodinium*) and Chlorophycea (*Chlamydomonas proboscigera*). Several OTUs affiliated with parasites such as Perkinsea, *Didymella* and *Cryptocaryon* were also found among the "victims" (Figure 1b).

Twenty-five OTUs displayed an increase in relative read abundance along with the chytrid epidemic. These OTUs were





Early beneficiary (4 OTUs):
Chytridiomycetes (Rhizophydiales, Lobulomycetales), Oligohymenophorea (*Stokesia*), Phyllopharyngea (Suctoria)

Late beneficiary (21 OTUs):
Chytridiomycetes (Rhizophydiales, Polychytriale, Chytridiales), Bacillariophyceae (*Fragilaria capucina*), Perkinsea, Chrysophyceae (*Dinobryon*, *Chromulina*, *Chrysonebula flava*), MAST (12C), Oligohymenophorea (Peniculia), Bicoecea (Bicosoecida), Cryptomycota (*Paramicrosporidium*), Oligotrichea (*Tintinnidium*, *Spirostrombidium*), Peronosporea (*Aphanomyces invadans*).

Victim (19 OTUs):
Bacillariophyceae (*Aulacoseira granulata*, *Asterionella formosa*, *Stephanodiscus agassizensis*), Chytridiomycetes (Spizellomycetales, Novel Clade II), Chrysophyceae (*Paraphysomonas*), Synurophyceae (*Mallomonas*), Prostomatea (*Cryptocaryon*), Oligotrichea (*Rimostrombidium*, *Spirostrombidium*, *Strombidium*), Dinophyceae (*Biecheleria*, *Symbiodinium*), Perkinsea (A31), Dothideomycetes (*Didymella*), Chlorophyceae (*Chlamydomonas proboscigera*).

Early opportunist (8 OTUs):
Chytridiomycetes (Rhizophydiales), Dinophyceae (*Gymnodinium*, *Amoebophrya*), Litostomatea (*Spathidium*), Heterotrichea (*Stentor*), Prostomatea (*Cryptocaryon*), Oligohymenophorea (*Telotrochidium*), Chrysophyceae.

Late opportunist (9 OTUs):
Chytridiomycetes (Chytridiales), Prostomatea (*Cryptocaryon*), Oligohymenophorea (*Vorticella*, *Epicarchesium*), Chrysophyceae (Monas, P34.45), Apicomonadea (*Colpodella edax*).

Neutral/Unclassified (20 OTUs):
Bacillariophyceae (*Aulacoseira granulata*), Synurophyceae (*Mallomonas punctifera*), Chrysophyceae (*Chrysosphaerella rotundata*), Trebouxiophyceae, Dinophyceae (*Scrippsiella hangoei*, Suessiaceae), Prostomatea (*Cryptocaryon*), Chrysophyceae, Peronosporea (*Aphanomyces*), Peronosporomycetes.

FIGURE 1 Dynamics of the microbial eukaryote community composition in Lake Pavin during the period of the diatom bloom—Chytrid epidemic. (a) Overall relative read abundance of main taxonomic groups throughout progression of the chytrid epidemic. (b) Succession patterns of each OTU based on their relative read abundance fluctuation (%). Standard deviations were calculated by considering relative read abundance of all OTUs within each category pattern ("beneficiary," "victim," "opportunist", and "neutral/unclassifed". Only OTUs having at least 50 reads in one sample were considered in this pattern analysis. OTU taxonomic affiliation is given at the class level and, in parentheses, at the most precise level.

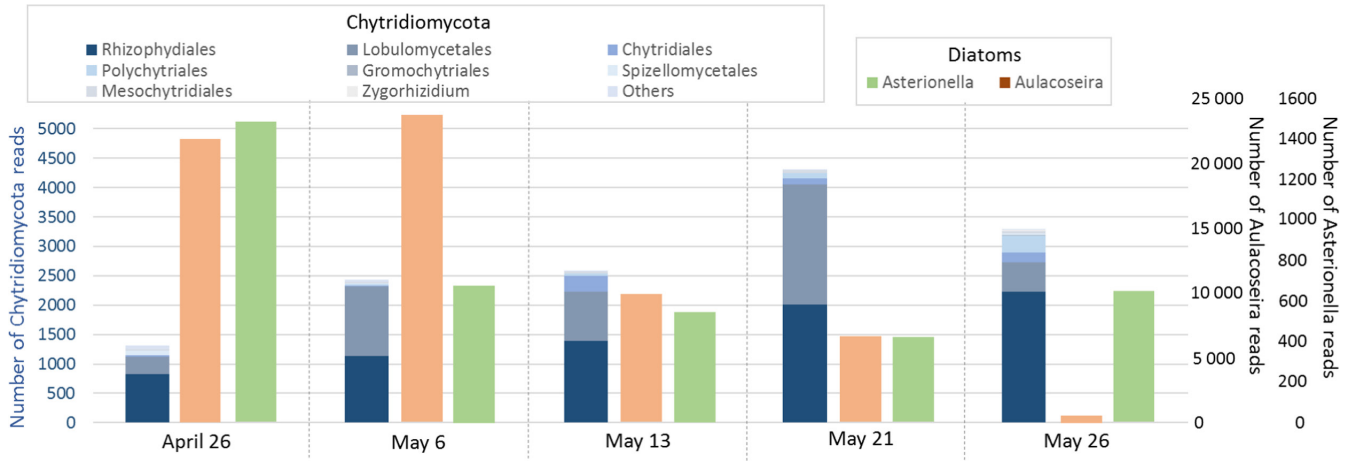


FIGURE 2 Read abundance of blooming diatoms and Chytridiomycota throughout progression of the chytrid epidemic. OTUs that belong to Chytridiomycota are represented by blue colour (different share corresponds to difference order), *Aulacoseira* is represented by red colour and *Asterionella* is represented by green colour.

TABLE 2 Diatom diversity during the chytrid epidemic and their potential fungal parasites

Host			Parasite
Host genera	**% of diatom reads**	**Found in Lake Pavin**[a]	**Known chytrid parasite genera**
Aulacoseira	92.4	*Au. granulata*[‡(§#)], *Au. subarctica*[‡(§)], *Au. crenulata*[†], *Aulacoseira* sp. 115[†]	*Zygorhizidium*, *Chytridium*
Asterionella	6.0	*As. formosa*[‡]	*Rhizophydium* (*planktonicum*), *Zygorhizidium*, *Zygophlyctis*, *Chytridium*
Fragilaria	0.4	*F. capucina*[†]	*Rhizophydium*, *Zygorhizidium*, *Chytridium*, *Podochytrium*
Stephanodiscus	0.4	*Ste. agassizensis*[‡]	*Rhizophydium*, *Zygorhizidium*
Melosira	0.2	*M. varians*[#]	*Rhizophydium*, *Zygorhizidium*, *Chytridium*, *Podochytrium*, *Rhyzidiopsis*, *Aphelidium*
Cymbella	0.01	*C. cistula*[†], *Encyonema minutum*[†]	*Chytridium*, *Rhizophydium*
Gomphonema	0.004	*Gomphonema* sp. TN-2014[§]	*Phlyctidium*, *Rhizophydium*
Navicula	0.003	*N. cryptotenella*[†]	*Rhizophydium*
Amphora	0.01	*Am. pediculus*[†]	*Physorhizophidium*
Pinnularia	0.008	*P. viridiformis*[#]	*Podochytrium*, *Rhizophydium*
Entomoneis	0.003	*Entomoneis* cf. *alata*[#]	—
Diatoma	0.1	*D. tenue*[‡(§#)], *D. vulgare* var. *linearis*[‡], *D. hyemalis*[‡]	—
Staurosirella	0.04	*Sta. pinnata*[†], *Staurosira* sp. D-20[†]	—
Planothidium	0.02	*Planothidium* sp. TF-2014[#]	—
Achnanthidium	0.02	*Ac. minutissimum*[†‡]	—
Epithemia	0.02	*Ep. turgida*[†]	—
Rhoicosphenia	0.02	*Rhoicosphenia* cf. *abbreviata* EWT-2016[#]	—
Sellaphora	0.005	*Sellaphora minima*[†]	—
Gomphoneis	0.005	*Gomphoneis minuta*[#]	—

[a]Host: [†]beneficiary, [‡]victim, [§]neutral, [#]others/opportunist: only tentative categories are given for genera having a low number of reads (<15 reads and representing <0.3% of diatom reads) as it is difficult to draw definitive conclusion for these OTUs.

classified as "early beneficiary" (four OTUs) or "late beneficiary" (21 OTUs) according to progression of the chytrid epidemic. Chytridiomycota (Rhizophydiales, Polychytriale, Chytridiale and Kappamycetaceae), other parasites (e.g., *Aphanomyces invadans*, Perkinsidae, *Paramicrosporidium*) and free-living small heterotrophs (e.g., MAST-12C, *Bicosoeca*) were also categorized as "beneficiary"

(Figure 1b). In addition, "beneficiary" also included OTUs that were affiliated with phytoplankton-grazing ciliates (Suctoria, *Stokesia*, *Tintinnidium*, *Peniculia*, *Spirostrombidium*), diatoms (*Fragilaria capucina*), golden algae Chrysophyceae (*Chromulina*, *Chrysonebula flava*) and mixotroph algae (*Dinobryon*). Two OTUs of *Paramicrosporidium* (Rozellomycota/Cryptomycota) appeared to have increased in read abundance with the progression of the chytrid epidemic and reached a significant peak on the last sampling date (Figure 1b). The category of (early or late) "opportunist" included OTUs that displayed fluctuating relative read abundances throughout the study period (with a sudden increase or decrease), suggesting temporary advantageous or detrimental conditions for these OTUs (Figure 1b). Among this category, 17 OTUs were affiliated with a variety of higher taxonomical groups such as Dinophyceae (*Gymnodinium*, *Amoebophyra*), Ciliates (*Spathidium*, *Stentor*, *Cryptocaryon*, *Telotrochidium*, *Vorticella* and *Epicarchesium*), Chrysophyceae (*Monas*) and Colpodellida (*Colpodella edax*). Finally, 20 OTUs were grouped in the "neutral/unclassified" category with read abundances being constant or displaying an unclear pattern of fluctuation during this period (Figure 1b).

3.1.2 | Succession and diversity during an epidemic event

Diatoms

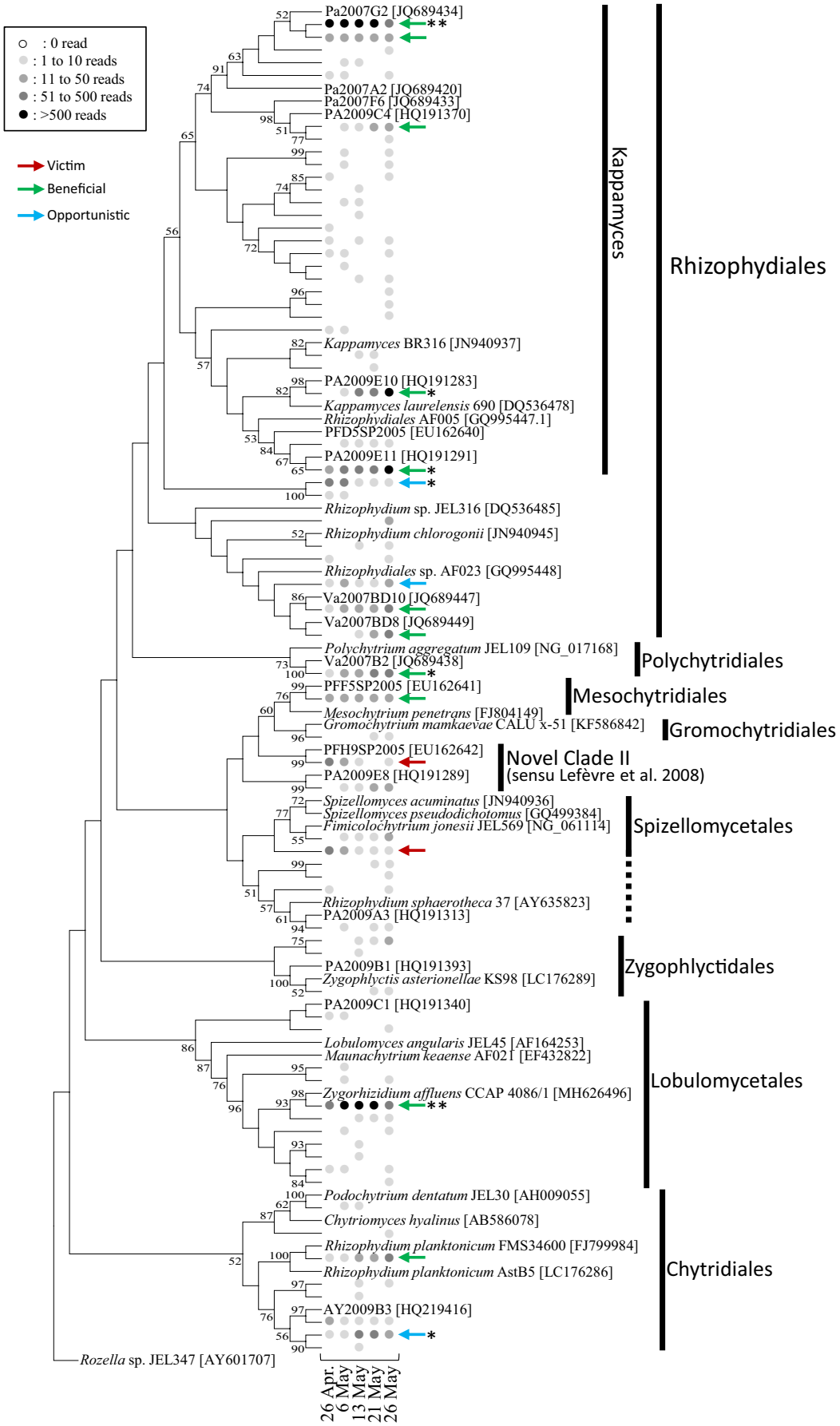
During the study period in Lake Pavin, a total of 120 OTUs affiliated to 26 diatom taxa and corresponding to 20.5% of all reads (metazoan excluded) were identified (Table 2). The diatom community was dominated by two species that represented over 98% of all diatom reads: *Au. granulata* and *As. formosa*. Note that among the most abundant OTUs (representing 98.8% of all reads), there were 10 OTUs of *Au. granulata* but only one OTU of *As. formosa*. Generally, it appeared that most diatoms were classified as either "beneficiary" or "victim" (Figure 1b, Table 2). A decrease in read abundances for *Au. granulata* and *As. formosa* was observed coincidentally with an increase in read abundance for Chytridiomycota (Figure 2). Thus, these two diatoms displayed a clear "victim" pattern of relative read abundance in relation to the progression of the chytrid epidemic (Figures 1b and 2).

Stephanodiscus agassizensis was also identified as a direct and/or indirect "victim" of the chytrid epidemic (Table 2). Other OTUs of diatoms were found in a low number of reads (representing <2% of all diatoms reads). Among them, *Diatoma tenu* was another potential "victim" of the chytrid epidemic, as suggested by its decreased relative read abundance, while *Fragilaria capucina* appeared to benefit from a decline in the *Asterionella/Aulacoseira* bloom, possibly due to a reduction in competition for resources (Figure 1b). *Gomphonema* sp. TN-2014 illustrated the "neutral" category by having a constant relative read abundance throughout the chytrid epidemic. Finally, *Melosira varians*, as well as many other "rare" diatoms, appeared to be "opportunists" with fluctuating relative read abundances (an increase during the early stage of the epidemic then a decrease at the late stage of the epidemic). This fluctuating pattern was also observed for some "victim" OTUs affiliated with *As. formosa*, *Au. granulata* and *Au. subarctica* (Table 2). However, due to low read numbers, such fluctuations cannot be conclusive.

Chytrids

During the study, 68 chytrid OTUs were identified, representing 4.5% of all reads (metazoans excluded). Cladogram analysis showed that the chytrid community was dominated by two OTUs that represented >60% of chytrid reads (indicated by "**" in Figure 3), while the seven most abundant chytridiomycetes OTUs corresponded to >90% of chytrid reads (indicated by "*" in Figure 3). The order Rhizophydiales dominated the chytrid community in terms of richness (35 OTUs) and read abundance (54%). Other major chytrid groups included Lobulomycetales (11 OTUs, 35% of chytrid reads), Chytridiales (eight OTUs, 4%) and Polychytridiales (one OTU, 3%). Metabarcoding data revealed a progression in the chytrid epidemic for each OTU (Figure 3), reaching its highest read abundance on May 21 (Figure 2). Most OTUs of Rhizophydiales, Chytridiales, Polychytridiales, Gromochytridiales and *Zygorhizidium* displayed an overall increase in read abundance during the study period. Considering the most abundant chytrid OTUs (with at least 50 reads, representing 97.7% of all chytrid reads), 11 out of 16 OTUs displayed the highest read abundance on May 21 or May 26. The most abundant chytrid OTU (representing 34% of all chytrid reads), affiliated with Lobulomycetales, increased in early May and peaked on May 21 (represented by a green arrow in Figure 3). The second most abundant chytrid OTU (representing 27% of all chytrid reads) also displayed a peak in reads on May 21 (green arrow in Figure 3), and was identified as a Kappamycetaceae in the order of facultative parasites Rhizophydiales. Nine other chytrid OTUs (together representing 29% of all chytrid reads) displayed increasing read abundances during the period (green arrows in Figure 3). Two OTUs affiliated with Spizellomycetales and "Novel Clade II" displayed an opposite pattern with read abundances decreasing over time (red arrows in Figure 3). Finally, three chytridiomycota OTUs (belonging to Chytridiales and Rhizophydiales) displayed higher read abundances shortly after decreases of diatoms (Figure 1b and blue arrows in Figure 3).

FIGURE 3 Chytridiomycetes affiliation based on cladogram analysis (with maximum likelihood) of OTU partial 18S rDNA sequences and chytrid reference sequences for precise taxon/clade assignments. For each OTU, succession is shown by individual circles (one for each sampling dates) with read abundance represented by the darkness of the circle (as shown in the key). Arrows indicate the most abundant chytrid OTUs (>50 reads) and their succession patterns ("beneficiary," "victim," "opportunist" and "neutral/unclassifed"), based on Figure 1b. Two asterisks (**) indicate the dominant OTUs (>3000 reads) and one asterisk (*) represents other abundant OTUs (>200 reads).


: 0 read
: 1 to 10 reads
: 11 to 50 reads
: 51 to 500 reads
: >500 reads
Victim
Beneficial
Opportunistic
Pa2007G2 [JQ689434]
Pa2007A2 [JQ689420]
Pa2007F6 [JQ689433]
PA2009C4 [HQ191370]
Kappamyces BR316 [JN940937]
PA2009E10 [HQ191283]
Kappamyces laurelensis 690 [DQ536478]
Rhizophydiales AF005 [GQ995447.1]
PFD5SP2005 [EU162640]
PA2009E11 [HQ191291]
Rhizophydium sp. JEL316 [DQ536485]
Rhizophydium chlorogonii [JN940945]
Rhizophydiales sp. AF023 [GQ995448]
Va2007BD10 [JQ689447]
Va2007BD8 [JQ689449]
Polychytrium aggregatum JEL109 [NG_017168]
Va2007B2 [JQ689438]
PFF5SP2005 [EU162641]
Mesochytrium penetrans [FJ804149]
Gromochytrium mamkaevae CALU x-51 [KF586842]
PFH9SP2005 [EU162642]
PA2009E8 [HQ191289]
Spizellomyces acuminatus [JN940936]
Spizellomyces pseudodichotomus [GQ499384]
Fimicolochytrium jonesii JEL569 [NG_061114]
Rhizophydium sphaerotheca 37 [AY635823]
PA2009A3 [HQ191313]
PA2009B1 [HQ191393]
Zygophlyctis asterionellae KS98 [LC176289]
PA2009C1 [HQ191340]
Lobulomyces angularis JEL45 [AF164253]
Maunachytrium keaense AF021 [EF432822]
Zygorhizidium affluens CCAP 4086/1 [MH626496]
Podochytrium dentatum JEL30 [AH009055]
Chytriomyces hyalinus [AB586078]
Rhizophydium planktonicum FMS34600 [FJ799984]
Rhizophydium planktonicum AstB5 [LC176286]
AY2009B3 [HQ219416]
Rozella sp. JEL347 [AY601707]
26 Apr.
6 May
13 May
21 May
26 May
Kappamyces
Rhizophydiales
Polychytridiales
Mesochytridiales
Gromochytridiales
Novel Clade II
(sensu Lefèvre et al. 2008)
Spizellomycetales
Zygophlyctidales
Lobulomycetales
Chytridiales

3.2 | Metatranscriptomic analyses

According to the results of metabarcoding, the samples of April 26 (diatoms showed the highest abundance) and May 21 (Chytridiomycota reached the highest abundance) were selected for metatranscriptomics analyses. A total of 360 million reads were generated after Illumina sequencing. After read processing and de novo assembly, a reference metatranscriptome was generated for mapping reads. Following differential expression analysis of read counts, a total of 50,819 transcripts that had more than twofold changes were identified, including 32,651 transcripts that were more abundant during the chytrid epidemic ("up-regulated" during the chytrid epidemic) and 18,168 transcripts that were more abundant during the diatom bloom ("down-regulated" during the chytrid epidemic). Among the 32,651 up-regulated transcripts, 5880 have $\log_2$ fold-change ≥10 and 15,072 transcripts have $\log_2$ fold-change ≥5. Among the 18,168 down-regulated transcripts, 221 have $\log_2$ fold change ≥10 and 3123 have $\log_2$ fold-change ≥5. After annotation using the BLASTX program, within up-regulated transcripts, 6966 transcripts of Chromista, 1214 Fungi, 1179 Protista, 12,026 Metazoa, 1240 Viridiplantae, 767 Bacteria, 11 Archaea, 15 viruses and nine uncultured organisms were identified; within down-regulated transcripts, 10,908 transcripts of Chromista, 156 Fungi, 187 Protista, 244 Metazoa, 260 Viridiplantae, 260 Bacteria, 12 Archaea, 24 viruses and three uncultured organisms were identified (Table 3). As expected, more transcripts of diatoms (Bacillariophyta) were down-regulated (9651) than up-regulated (852) during the chytrid epidemic. For Chytridiomycota, more transcripts were up-regulated (546) than down-regulated (56) during the chytrid epidemic.

3.2.1 | Active functions in Chytridiomycota during chytrid epidemics

According to the results of BLASTX analyses, putative functions of transcripts were assigned. During the chytrid epidemic, functions of 546 up-regulated transcripts in Chytridiomycota were identified (Table 4). They could be generally categorized as: 69 stress-tolerance-related, 17 host-invasion-related, 45 host nutrient processing, 52 colonization-related, 342 other cell functions and 21 unknown/hypothetical proteins. Around one-third of these transcripts appeared relevant to the parasite lifestyle. Stress-tolerance-related functions include proteins in response to reactive oxygen species (ROS), heat/cold-shock proteins, chaperons, and DNA damage-repair proteins. Host-invasion-related functions include cell adhesions, toxins and proteases/peptidases. Functions of host nutrient processing are largely related to the proteasome and ubiquitin for degrading and recycling host proteins. Colonization-related functions include tubulin/microtubule forming, morphological changes, organelles forming, and cell division. Note that the above colonization-related functions can also contribute to sporangia and zoospore development. Among the 342 other cell functions, 30 were related to carbohydrate metabolism, 35 to amino acid/protein metabolism, 15 to nucleic acid metabolism/processing, and 21 to fatty acid/sterol metabolism (Table 4). Even though some functions may not be directly related to parasitism, such as ribosomal proteins (111 transcripts), transporters (29 transcripts), energy production (23 transcripts) and cellular messaging (24 transcripts), these are essential functions to maintain healthy and active living cells (Table 4).

TABLE 3 Summary of BLASTX annotation for metatranscriptomics analyses

Number of up-regulated genes								
	During diatom bloom				During chytrid epidemic			
Fold-change ($\log_2$)	≥10	<10, ≥5	<5, ≥1	Subtotal	≥10	<10, ≥5	<5, ≥1	Subtotal
Archaea	0	5	7	12	4	4	3	11
Bacteria	8	64	188	260	103	409	254	766
Chromista (Bacillariophyta)[a]	94 (11)	1472 (988)	9339 (8652)	10,905 (9651)	1380 (36)	3049 (108)	2526 (708)	6955 (852)
Fungi (Chytridiomycota)[b]	3 (0)	40 (4)	113 (61)	156 (56)	71 (12)	329 (71)	793 (463)	1193 (546)
Metazoa	11	47	186	244	2111	5722	4192	12,025
Protista	7	82	98	187	134	829	215	1178
Viridiplantae	5	82	173	260	152	628	459	1240
Virus	3	11	10	24	2	4	9	15
Unknown	79	328	4001	6120	1875	3919	2077	9268
Total	221	3123	14,826	18,168	5880	15,072	11,699	32,651

[a]Numbers in parentheses represent the number of up-regulated genes that were identified as Bacillariophyta genes by the BLASTX analysis. Bacillariophyta (diatoms) is a phylum in the kingdom Chromista.

[b]Numbers in parentheses represent the number of up-regulated genes that were identified as Chytridiomycota genes by the BLASTX analysis. Chytridiomycota (chytrids) is a phylum in the kingdom Fungi.

TABLE 4 Number of up-regulated Chytridiomycota functions during the diatom bloom and the chytrid epidemic

Function	During diatom bloom	During chytrid epidemic
Stress tolerance-related		
Chaperone, stress response proteins	8	36
Cytochrome	1	8
DNA repair and DNA metabolism, SUMOylation of DNA damage response and repair proteins	0	2
Response to reactive oxygen species, destroys radicals, cell redox homeostasis-related	0	23
Host invasion-related		
Cell adhesion-related	0	3
Formation of pigments such as melanins and other polyphenolic compounds	0	1
Nonribosomal peptide synthetase	0	1
Proteases, peptidase	0	11
Septin	0	1
Host nutrient processing-related		
Ubiquitin, polyubiquitin, proteasome	2	45
Colonization-related		
Actin/tubulin-related, microtubule-associated	13	35
Cell cycle control, cell division, cell projection morphogenesis-related	0	7
Cilia- and flagella-associated	3	6
Organelle-forming and morphological change-related	0	4
Cellular messaging/signalling-related		
14–3-3 signalling pathway	0	4
Calcium-modulated protein	0	3
cAMP and cGMP related	0	3
GTP binding protein	0	4
Signal transduction	1	10
Metabolism-related		
Amino acid and protein metabolism	1	35
Carbohydrate metabolism	1	30
Lipid/fatty acid metabolism	0	21
Metabolism of secondary metabolites, cofactors, hormones, large, hydrophobic compounds	0	6
Nucleotide/nucleic acid metabolism	0	15
Others		
Channel, transporter, ion homeostasis	7	29
Energy generation/transformation, ATP biosynthetic process, mitochondrial proteins	1	23
Histone	5	12
Methylation reaction-related	3	0
Ribosomal proteins	9	111
rRNA	4	0
Translation initiation/elongation factors	3	20
Vesicle trafficking, protein transport, endoplasmic reticulum, endosome-related	0	16
Unknown/hypothetical proteins	3	21
Total	65	546

TABLE 5 Number of up-regulated diatom functions during the diatom bloom and the chytrid epidemic

Diatom function	During diatom bloom	During chytrid epidemic
Actin, tubulin, cytoskeleton-related	41	9
Caspase/metacaspase	15	0
Channels, transporters	317	7
Chaperone, stress response proteins	130	16
Chitinase	14	10
DNA or RNA polymerase and associated protein	32	2
Fucoxanthin, carotenoid-related	19	87
Histone-related	75	2
Lipase	28	0
Lipid/fatty acid metabolism	41	6
Peroxisome-related	49	5
Photosystem-related	10	18
Protease	237	14
Proteasome, ubiquitin	121	7
Ribosomal proteins	272	52
Vacuolar-related	62	2
Others functions	5615	358
Unknown/hypothetical proteins	2588	257
Total	9651	852

3.2.2 | Active functions in diatoms

Full genome sequences of *Asterionella* and *Aulacoseira* are unavailable. Therefore, it is difficult to indicate which active functions belonged to these two diatoms. Among the few diatom species with whole genome sequences available to date, *Phaeodactylum* and *Thalassiosira* are the most well-studied genera and with more information on annotated genes in databases. Hence, it is not surprising that the majority of diatom functions matched gene homologues in *Phaeodactylum* or *Thalassiosira* after BLASTX searches. Nonetheless,

neither *Phaeodactylum* nor *Thalassiosira* appeared in our metabarcoding data. Since the diatom community was dominated by *Au. granulata* and *As. formosa* (representing over 98% of all diatom reads), it is highly likely that diatom transcripts abundantly expressed during the spring bloom (9651 transcripts, Table 5) represented active functions of these two diatoms. Comparatively, transcripts/functions involving lipases, transporters, histone, vacuolar systems, proteasome, proteases, DNA/RNA polymerases, and caspases/metacaspases were more abundant during the diatom bloom (Table 5). On the other hand, during the chytrid epidemic, abundantly expressed diatom transcripts (852 transcripts, Table 5) could come not only from defensive functions of *Asterionella* and *Aulacoseira*, but also from active functions of other diatoms that were categorized as "beneficiary" of chytrid epidemics. Transcripts/functions involving fucoxanthin, photosystem, chitinase, actin/tubulin, ribosomal proteins and fatty acid metabolism were relatively more abundant during the chytrid epidemic (Table 5).

3.2.3 | Active functions in "beneficiaries" during chytrid epidemics

In addition to active functions in diatoms, the functions of other "beneficiary" organisms during chytrid epidemics were also extensively explored based on BLASTX results and available genome information. A diverse ciliate protozoa class, Spirotrichea, had 1658 transcripts identified as more abundant during the chytrid epidemic (Table 6). Besides ribosomal proteins and hypothetical proteins, transcripts of protease/peptidase (including proteasome-related protease and ubiquitin) were particularly abundant (176 transcripts, Table 6). Other abundant functions include 54 energy/ATP-related transcripts, 48 carbohydrate metabolism-related and 11 lipid metabolism-related (Table 6). The abundance of functions related to protein, carbohydrate and lipid metabolism and energy suggests that "beneficiary" Spirotrichea were active and possibly consuming chytrid zoospores. Together, these metatranscriptomics results agree with the metabarcoding results and suggest that these Spirotrichea benefited from new food sources rich in protein, carbohydrate and fatty acids during the chytrid epidemic. Above all, this could be an example of a "beneficiary" profiting from the "trophic upgrading" phenomenon prompted by chytrid epidemics.

TABLE 6 Up-regulated functions in Spirotrichea during the chytrid epidemic

Function	Number of up-regulated transcript
Actin, tubulin, cytoskeleton-related	40
ATPase/ATP synthase	54
Channels, transporters	21
Carbohydrate metabolism	48
Cytochrome	21
Dynein	24
Kinase	52
Lipase/fatty acid metabolism	11
Protease/peptidase, proteasome, ubiquitin-related	176
Ribosomal proteins	159
Other functions	788
Unknown/hypothetical proteins	264
Total	1658

3.2.4 | A panorama of active functions in a eukaryotic microbial community

One major advantage of metatranscriptomics is that it provides a snapshot of all active functions in a microbial community at the time of sampling. During the diatom bloom, 12,062 up-regulated transcripts had probable functions identified. During the chytrid epidemic, 23,427 up-regulated transcripts had probable functions identified. Generally categorized by functions, details of these transcripts are presented in Table 7. Comparing the two sampling points, a higher proportion of up-regulated transcripts were categorized as hypothetical/uncharacterized proteins during the diatom bloom, with most of them belonging to Bacillariophyta (diatoms). This is probably due to the lack of complete *Asterionella* and *Aulacoseira* genome sequences in the database. Even though a few diatom species have genome sequences available to the scientific community, not all of them are fully annotated or functionally characterized. During the diatom bloom, transcripts that were categorized as transporters/ion channels, thioredoxin, clathrin and flavodoxin were found at a higher proportion; functions related to signal transduction networks and ATPase were found at a slightly higher proportion. During the chytrid epidemic, up-regulated functions related to ribosomal proteins, cytoskeleton, mitochondrial proteins, cellular cargo/vesicle transportation, ubiquitin/polyubiquitin, photosynthesis, ferritin/ferredoxin and flagellar were found at higher proportions (Table 7). Although functions of metabolic enzymes were found in comparable proportions during the diatom bloom and the chytrid epidemic, interestingly, almost one-fifth of metabolic enzymes during the chytrid epidemic were protease/peptidase. In addition, when taking the function of ubiquitin/polyubiquitin into account, during the chytrid epidemic, 6.81% of identified transcripts were related to the function of protein degradation in contrast to 4.80% during the diatom bloom. Similarly, functions of lipid metabolism and chitinase were found at higher proportion during the chytrid epidemic (1.02% and 0.17% respectively) than during the diatom bloom (0.52% and 0.07% respectively). To support the parasite lifestyle, chytrids highly express functions of protein degradation including protease, proteasome and ubiquitin to process nutrients from the host. Still, according to both metabarcoding and metatranscriptomics results, chytrids comprised only a small part of the total population in the community. The majority of protein-degrading functions in the community were expressed by nonchytrid organisms, especially by protist Intramacronucleata (including the above-mentioned "beneficiary Spirotrichea") and metazoa (e.g., Nematoda and Rotifera). These protein-degrading functions were probably expressed by such organisms to process ingested food: chytrid zoospores.

TABLE 7 Number of up-regulated functions in the eukaryotic microbial community during the diatom bloom and the chytrid epidemic

	During diatom bloom		During chytrid epidemic	
Eukaryotic microbial function	**Number**	**% of total**	**Number**	**% of total**
ATPase	203	1.68	354	1.51
ATP-binding cassette	13	0.11	55	0.23
Caspase/metacaspase	17[a]	0.14	5	0.02
Cellular cargos/vesicle transportation	189	1.57	653	2.79
Chitinase	8	0.07	39	0.17
Clathrin	24	0.20	26	0.11
Cytochrome	56	0.46	265	1.13
Cytoskeleton-related	239	1.98	1158	4.94
Fasciclin (cell adhesion)	7	0.06	30	0.13
Ferredoxin	5	0.04	24	0.10
Ferritin	1	0.01	22	0.09
Flagellar	24	0.20	115	0.49
Flavodoxin	4	0.03	1	0.00
Glutathione-related	45	0.37	103	0.44
Histone	111	0.92	217	0.93
Light harvesting and photosystem-related	50	0.41	258	1.10
Lipid metabolism	63	0.52	238	1.02
Metabolic enzymes (without lipase, protease, peptidase)	2766	22.93	5282	22.55
Mitochodrial proteins	181	1.50	939	4.01
Nucleic acid replication, transcription and translation-related	582	4.83	1045	4.46
Others functions	2223	18.43	4068	17.36
Protease/peptidase	426	3.53	1098	4.69
Ribosomal proteins	535	4.44	2370	10.12
Ribosylation-related	18	0.15	58	0.25
Senescence-associated protein	4	0.03	74[b]	0.32
Signal transduction network-related	520[c]	4.31	972	4.15
Stress response/chaperon proteins	281	2.33	595	2.54
Thioredoxin-related	44[a]	0.36	69	0.29
Transmembrane protein	40	0.33	171	0.73
Transporters/ion channels	366	3.03	342	1.46
Ubiquitin/polyubiquitin	153	1.27	497	2.12
Unknown/hypothetical proteins	2864	23.74	2284	9.75
Total	12,062		23,427	

[a]Mostly from diatoms.

[b]Not from diatoms or from chytrids.

[c]Mostly not from diatoms nor from chytrids.

4 | DISCUSSION

4.1 | Dynamics in eukaryotic microbial biodiversity

The chytrid epidemic is a massive event in Lake Pavin expected to impact microbial community succession directly or indirectly. Different patterns of relative read abundances could reflect not only host–parasite competition, but also influences on members of the community (e.g., "beneficiary," "victim" and "opportunist") along with progress of the bloom epidemic. Among affected members, *As. formosa* is a diatom that blooms in Lake Pavin and a host for the chytrid parasite (Sime-Ngando, Gerphagnon, et al., 2016). Although chytrid infection in *Aulacoseira* has not been reported in Lake Pavin, previous studies have shown that *Aulacoseira* could be a host for parasitic chytrids (Kagami et al., 2012; Maier & Peterson, 2014; Seto & Degawa, 2018). *Fragilaria crotonensis* was reported previously as a host for chytrids (Canter & Lund, 1953), but *F. capucina* (identified in this study, Table 2) has not been reported to be

infected by chytrids. *Stephanodiscus agassizensis* was identified as a "victim" of the chytrid epidemic (Table 2), although only *S. parvus* has been previously reported to be parasitized by the *Zygorhizidium* sp. in Lake Schohsee, Germany (Holfeld, 1998). *Diatoma tenu* appeared to be another potential "victim" of the chytrid epidemic, but its infection by chytrids has not yet been reported in the literature.

It is of note that two OTUs of *Paramicrosporidium* (Rozellomycota/Cryptomycota) appeared to be "beneficiaries" of the chytrid epidemic (Figure 1b). Rozellomycota/Cryptomycota were previously described as having mycoparasitism ability and could be parasites or hyperparasites of chytrids (Grossart et al., 2019). However, the roles of these two OTUs in the community of Lake Pavin during the chytrid epidemic are unclear. Further investigation will be needed to clarify whether other hidden players are involved in the host–parasite relationship between diatoms and chytrids.

The variation in chytrid 18S rDNA sequences of the present study (Figure 3) along with the results of previous studies during chytrid epidemics in Lake Pavin (Lefèvre et al., 2007, 2008; Monchy et al., 2011) showed that a reservoir of chytrid diversity is present in the lake. This chytrid reservoir cannot be in the lake sediment because Lake Pavin is a deep, meromictic lake which has layers of water that do not intermix for decades or centuries (Sime-Ngando, Boivin, et al., 2016). Further investigation will be needed to locate the chytrid reservoir in Lake Pavin.

4.2 | Metatranscriptomic results reflected activities in the community

4.2.1 | Chytrids

Metatranscriptomic analyses of a community at different times provided a glimpse into gene expression dynamics in situ (Salazar et al., 2019). During the chytrid epidemic, chytrid transcripts related to invasion, host nutrient processing and colonization were up-regulated according to metatranscriptomics results (Table 4). Parasitic chytrids need to invade diatom frustules and extract nutrients to support colonization (Canter-Lund & Lund, 1995). Host-invasion-related functions include cell adhesions, toxins and proteases/peptidases (Alberts et al., 2002). Functions of host nutrient processing are largely related to proteasome and ubiquitin for degrading and recycling host proteins (Alberts et al., 2002). Colonization-related functions include tubulin/microtubule formation, morphological changes, organelle forming and cell division (Alberts et al., 2002). These colonization-related functions can also contribute to sporangia and zoospore development. At the same time, stress-tolerance-related functions including response to ROS, heat/cold-shock proteins, chaperones and DNA damage-repair proteins are also elevated in chytrids (Table 4). When encountering infections, ROS are rapidly produced by host cells for defence (Jajic et al., 2015). Therefore, it is rational for chytrids to up-regulate stress-tolerance functions to overcome oxidative stress. Additionally, up-regulated functions related to metabolism of carbohydrates, amino acids, fatty acids and nucleic acids in chytrids (Table 4) are probably due to developments of zoospores as chytrid zoospores are rich in carbohydrates, proteins, nucleic acids, sterols and diverse fatty acids (Weete et al., 2010).

4.2.2 | Diatoms

During the diatom bloom, abundantly expressed diatom transcripts were probably from *Aulacoseira* and *Asterionella* since over 98% of all diatom reads belonged to these two genera according to metabarcoding results. Besides transcripts representing cellular functions of diatoms, notably a higher proportion of caspases/metacaspases were up-regulated (Table 5). Caspases and metacaspases are known to induce programmed cell death (Elmore, 2007). A form of programmed cell death, the hypersensitivity response, is one of the strategies that hosts can use against parasite attack (Balint-Kurti, 2019). In *As. formosa*, a possible hypersensitivity response has been previously described with a quick death of host cells shortly after infection (Canter-Lund & Lund, 1995). Up-regulation of caspases/metacaspases could be an early sign of chytrid infection in diatoms, but it could be also induced by overpopulation due to the bloom. Further studies are needed to clarify this.

Notably, during the chytrid epidemic, abundantly expressed diatom transcripts were not just from *Asterionella* and *Aulacoseira*. Fucoxanthin-related functions were significantly up-regulated in diatoms during the chytrid epidemic and one of the transcripts even had >11,346-fold increases. According to previous studies, the fucoxanthin/chlorophyll ratio in *As. formosa* is quite stable (Kirk, 1994; Zastrow, 2001). However, only functions involved in fucoxanthin production increased but not functions involved in chlorophyll production during the chytrid epidemic. Therefore, increasing fucoxanthin-related transcripts was probably expressed from species other than *As. formosa* (such as "beneficiary" diatoms). Another up-regulated diatom function was chitinase (Table 5). Chitin is the second most abundant polymer on Earth and both chytrid (fungus) and diatoms are known chitin producers (Durkin et al., 2009). During the chytrid epidemic, Bacillariophyta chitinases could be transcribed by *Asterionella and Aulacoseira* to defend against chytrid infection. However, chitinases could also be transcribed by "beneficiary" diatoms to digest chytrids for nutrients or simply for diatom cell wall restructuring (Durkin et al., 2009). Similarly, increasing transcripts of the photosystem, actin/tubulin, ribosomal proteins and fatty acid metabolism could be also due to the growth of "beneficiary" diatoms. Associating transcripts with diatom species could be improved in the future when full genomes of more diatom species become available.

4.3 | A global view

During the diatom bloom, up-regulated functions related to thioredoxin, clathrin and flavodoxin were found in higher proportions, while functions related to ferritin/ferredoxin were in lower

proportions (Table 7). As the bloom progressed, the community could be confronted with situations such as oxidative stress, nutrient availability and iron limitation (Behrenfeld & Kolber, 1999). During iron limitation, diatoms are likely to substitute iron-containing enzymes/proteins with functionally equivalent, noniron-containing ones, for example substitute ferredoxin with flavodoxin (Allen et al., 2008; McKay et al., 1997). Similar signs were also observed in previous studies of iron limitation during diatom blooms (Deana et al., 1999; Marchetti et al., 2012; van Creveld et al., 2014). Although in previous reports expressions of flavodoxin in *Fragilariopsis cylindrus*, *Cylindrotheca closterium* and *Thlassiosiroid pseudonana* appeared not to be sensitive to iron concentration (Pankowski & McMinn, 2008; Whiteley et al., 2017), these species were not detected in our metabarcoding data. Therefore, we cannot rule out the possibility that at the sampling time of the diatom bloom in Lake Pavin, the community could be confronted with iron limitation. On the other hand, according to our metatranscriptomics data during the chytrid epidemic, higher proportions of iron-containing proteins were up-regulated, including cytochromes, ferredoxin and ferritin, but not flavodoxin (Table 7), suggesting iron was no longer limited. Further sampling and investigation of iron concentrations in Lake Pavin throughout this period should clarify the question of whether the community was confronted with iron limitation during the spring diatom bloom and whether the chytrid epidemic relieved the iron limitation.

Overall, in the community during the chytrid epidemic, functions related to degradation/metabolism of proteins, lipids and carbohydrates were up-regulated (Table 7), suggesting community members' intake of food was rich in protein, lipid and carbohydrates (which resembles chytrid zoospores; Kagami et al., 2014, 2017; Weete et al., 2010). Our gene expression results seem to agree with the "trophic upgrading" scenario of "nutrient transfer from diatom hosts to the zooplankton community through chytrid zoospores" in "mycoloop" theories (Agha et al., 2016; Kagami et al., 2014).

Besides serving as a nutrient supplement, ingested chytrid zoospores may also become part of gut microbiota for some community members since ingested food is a source of microorganisms for gut microbiota (Sommer & Bäckhed, 2013). Would the location of the above-mentioned chytrid diversity reservoir include gut microbiota? In aquatic ecosystems, it has been shown that fungi play a part in the gut microbiota of many insect larvae such as black flies and Amazonian shredder insects (Belmont-Montefusco et al., 2020; McCreadie et al., 2011). Although beyond the scope of this current study, this could be an interesting subject for a future project.

5 | CONCLUSION

In this study, we investigated the host–parasite interactions in Lake Pavin (France) during the spring diatom bloom and the following chytrid epidemics using NGS. Eukaryote microbial biodiversity, community structure and the peak of chytrid infection in diatoms were determined using metabarcoding analyses. Besides diatoms, other potential "beneficiaries" and "victims" of the chytrid epidemic were also suggested. Subsequently, metatranscriptomic analyses were applied to reveal active functions in the microbial community during the host–parasite interactions. Diatom functions involving lipases, transporters, histone, vacuolar, proteasome, proteases and DNA/RNA polymerases were more abundant during the diatom bloom. The eukaryotic microbial community in Lake Pavin might experience nutrient limitation during the diatom bloom. Chytrid functions related to a parasitic lifestyle including invasion, colonization and stress tolerance were up-regulated during the chytrid epidemic. Functions related to degradation/metabolism of proteins, lipids and chitin were in higher proportion in the community during the epidemic, especially from "beneficiary" protists and metazoa. Our results support the view that chytrid zoospores can be consumed by community members in the lake and represent a nutrient source supplementing the food web. For future studies, extensive long-term sampling/monitoring along with palaeolimnological and molecular biology analyses could be a suitable strategy to elucidate details of food web interactions, trophic dynamics and contributions to biogeochemical cycles.

AUTHOR CONTRIBUTIONS

T.S.-N., E.V., U.C. and S.M. conceived the study; M.G. conducted lake water sampling; P.D.-V. performed nucleic acid extraction; L.-L.L. and S.M. performed data analyses, interpreted the results and wrote the manuscript.

ACKNOWLEDGEMENTS

This work was financially supported by the French "Agence Nationale de la Recherche" (ANR-12-BSV7-009-01) and "Contrat de Plan État-Région (CPER)" under projects MARCO and IDEAL. We thank the reviewers for their constructive comments that greatly improved this work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT AND BENEFIT-SHARING

Raw Illumina sequence reads from metabarcoding and metatranscriptomics are deposited in the NCBI Sequence Read Archive under BioProject no. PRJNA789723.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Li, L.-L., Delgado-Viscogliosi, P., Gerphagnon, M., Viscogliosi, E., Christaki, U., Sime-Ngando, T., & Monchy, S. (2022). Taxonomic and functional dynamics during chytrid epidemics in an aquatic ecosystem. *Molecular Ecology*, *31*, 5618–5634. https://doi.org/10.1111/mec.16675