TEMPORAL VARIATION GOVERNS THE PROLIFERATION OF DIFFERENT TAXA OR GUILDS OF ULTRAPLANKTONIC FUNGI IN A LARGE RESERVOIR

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Abstract. The fungus play an important role within the aquatic ecosystem. However, the ultraplanktonic fungus diversity in reservoir ecosystems is still greatly unexplored. In this study, we used Illumina sequencing technology to analyze the diversity of ultraplanktonic fungi (size class 0.7–5 μm in this study) from two sampling sites along the Three Gorges Reservoir of the Yangtze River in China at four seasons in 2015. *Basidiomycota* was the most abundant phylum (36.69% of the total fungal reads), followed by *Ascomycota* (27.05%), *Cryptomycota* (18.06%), *Chytridiomycota*, (15.81%) and *Blastocladiomycota* (1.51%). Our study showed that ultraplanktonic fungi were changing with time, and a shift in dominant taxonomic groups was observed between time points. A marked temporal variation in functional guilds of fungi was also observed, suggesting that temporal variation governs the proliferation of different taxa or guilds of fungi in the reservoir. Our results suggest that inputs from fungal species of terrestrial origin may be responsible for the observed changes in pelagic community composition. This result also implies that terrestrial inputs may be an important source of fungal species in reservoir ecosystems. Additionally, it is possible that the chytrids were limited by hyperparasitic *Cryptomycota*.
Keywords: *reservoir ecosystem, aquatic fungi, high-throughput sequencing, diversity and distribution, functional guilds*

Introduction

Fungi represent one of the last frontiers of the unexplored biodiversity that challenges microbial ecology today (Lepère et al., 2018). The kingdom of fungi is highly diverse. Recent estimates indicate that the number of aquatic fungal species lies between 3000 and 4000 (Grossart and Rojas-Jimenez, 2016). Aquatic fungi have been detected from a wide range of habitats, including the ocean (Pachiadaki et al., 2016; Hassett et al., 2019), coastal regions (Gao et al., 2010; Duan et al., 2018), lakes (Lefèvre et al., 2012; Comeau et al., 2016; Wahl et al., 2018), and rivers (Duarte et al., 2015; Seena et al., 2019). However, relatively few studies have investigated the biodiversity and ecological role of mycoplankton in reservoir ecosystems.

Fungi play an important role in aquatic ecosystems. They influence and shape them by cycling organic matter and channeling nutrients across trophic levels (Nilsson et al.,

2019). Some fungi decompose leaves and wood (Canhoto et al., 2017), and others are important sources of food and nutrients for filter-feeding zooplankton and copepods (Rasconi et al., 2011; Sime-Ngando et al., 2011; Frenken et al., 2018). Moreover, rather than just serving as prey and a source of nutrients, fungi have been found to potentially establish alternative trophic links between large inedible phytoplankton and zooplankton through the "mycoloop" pathway (Kagami et al., 2014; Agha et al., 2016; Gerphagnon et al., 2019).

The majority of aquatic fungi occur in freshwaters (Shearer et al., 2007). As threats to freshwater biodiversity escalate, there is an urgent need to survey, collect and isolate freshwater fungal species. Most aquatic fungi are microscopic (Wurzbacher et al., 2011), hence ultraplanktonic fungi have a major role in aquatic environments. Despite their ecological importance, ultraplanktonic fungi have remained poorly described because of their small size and lack of morphological characteristics. Therefore, it is important to understand ultraplanktonic fungi diversity in freshwater ecosystems. In some studies, culture-independent molecular techniques have been exclusively used to determine fungal diversity in freshwater habitats. Several studies have surveyed the composition, distribution, and function of planktonic fungi in reservoir ecosystems (Chen et al., 2018). Unfortunately, most studies of large freshwater systems are conducted at one time points. For diversity studies, the inclusion of time surveys allows for the detection of a wider diversity than time-point studies (Simon et al., 2015). Nevertheless, knowledge is scarce regarding temporal series diversity of ultraplanktonic fungi in large reservoir ecosystems. Lack of information on ultraplanktonic fungi in large reservoir ecosystems limits the comprehensive understanding of aquatic ecosystems.

The Three Gorges Reservoir is located in the upstream reaches of Yangtze River in China. Presently, it is one of the largest reservoirs in the world (Han et al., 2015). In this study, a high-throughput Illumina sequencing technology was used to analyze the diversity of ultraplanktonic fungi (size class 0.7–5 μm in this study). Two sites were considered along the Three Gorges Reservoir of the Yangtze River and sampled over four seasons in 2015. The objective of our study was to provide a taxonomic and functional characterization of the ultraplanktonic fungal community of a large reservoir at two diametrically opposed sites on 4 occasions during the year.

Materials and methods

Sampling and measurement of physico-chemical parameters

In total, eight water samples were collected at two sites (CJ01 and CJ05) from the Three Gorges Reservoir of the Yangtze River of China in the following months of 2015: April (spring), August (summer), October (autumn), and December (winter) (*Fig. 1*). The Three Gorges Reservoir is a canyon-shaped, typical river-type reservoir. The water body type of the two sample sites were the same. These sites are in a transition-type region (Zheng et al., 2006). The sampling sites were located in the middle of the river; CJ01 (E 110.99990, N 30.82111) is approximately 1.6 km and CJ05 (E 110.84682, N 30.88436) is approximately 200 m away from the riverbank. The sampling site CJ01 was located approximately 1 km upstream of the Three Gorges Dam.

Water samples were collected from a depth of about 0.5 m beneath the water surface by using a 5-L sampler. These samples were pre-filtered through 5-μm pore-size polycarbonate filters (Millipore, USA). Then, these samples were filtered through GF/F

filters (0.7 µm, Whatman). The filters were immediately frozen in liquid nitrogen and stored at –80 °C until further analysis.

Physico-chemical factors, such as water temperature (WT), dissolved oxygen (DO), pH, and turbidity, were measured *in situ* with an YSI model Professional Plus multiparameter probe (YSI, USA). According to standard methods described in previous studies, we determined the following parameters: chemical oxygen demand (COD), oxidation-reduction potential (ORP), the concentrations of total nitrogen (TN), orthophosphate (PO_4^{3-}), nitrate (NO_3^-), total phosphorus (TP), and ammonium (NH_4^+) (Huang et al., 1999). Trophic states of different sampling sites and times were determined from comprehensive nutritional status index (trophic level index, TLI) by using the values of following parameters: TN, TP, and COD (Tian et al., 2017). Trophic states were set as follows: $TLI \le 30$, oligotrophic; $30 < TLI \le 50$, mesotrophic; $50 < TLI \le 60$, slightly eutrophic; $60 < TLI \le 70$, moderately eutrophic; $TLI > 70$, highly eutrophic (Meng and Zhao, 2007).
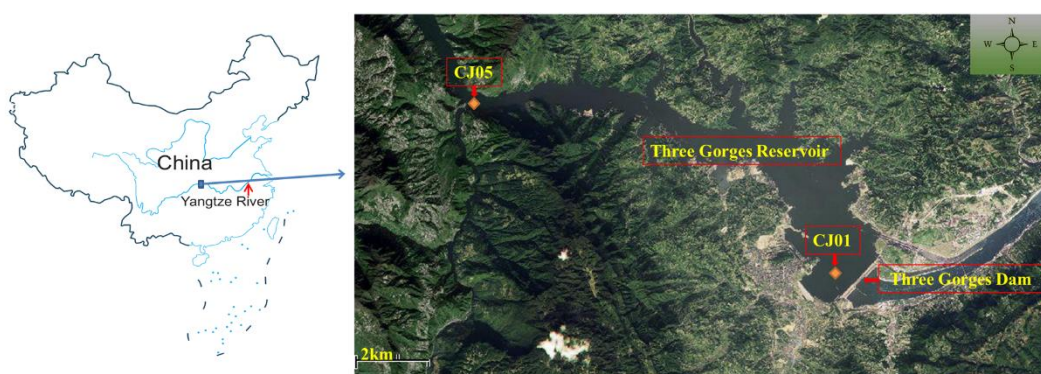

Figure 1. *Map of the study area and sampling site*

DNA extraction, amplification, and sequencing of 18S rDNA fragments

A previously described method was used to extract DNA from filters (Countway et al., 2005). To amplify the 18S small subunit rRNA gene fragments from samples, we used the following fungal-specific primers: 817F (5′-TTAGCATGGAATAATRRAATAGGA -3′) and 1196R (5′-CCTTTGAGTGGTCCAGGTCT -3′) (Borneman and Hartin, 2000). To modify primer sequences, Illumina adaptor was added to the ends of the forward primer and another adaptor was added to the ends of the reverse primer. After amplification, the amplicons were purified directly by using UNIQ-10 PCR purification kit (Sangon Biotech Ltd, Shanghai, China); the purified amplicons were quantified with TBS-380 (Turner BioSystems Inc, USA). Paired-end sequencing of purified amplicons was carried out on an Illumina MiSeq PE250 platform (Majorbio Bio-Pharm Technology Ltd, Shanghai, China).

Sequence processing

The raw demultiplexed reads were first quality trimmed Mothur software version 1.28.0 (Schloss et al., 2009). Following these filtering parameters, some reads were removed due to quality considerations: the length of nucleotides was less than 300 bp; it contained ambiguous bases, which were imperfectly matched with a sample-specific

barcode; and the average sequencing quality scores were less than 25 for the whole sequence. Potentially chimeric sequences were identified and removed using UCHIME (Edgar, et al., 2011). By using the furthest neighbour method (http://www.mothur.org/wiki/Cluster), we clustered unique sequences into operational taxonomic units (OTUs) at 97% sequence identity. Subsequently, a representative sequence from each OTU was aligned against the SILVA 123 reference alignment using the RDP classifier (Wang et al., 2007). The functional group (guild) of fungal OTUs was determined by using FUNGuild v1.0 database (Nguyen et al., 2016).

Statistical analyses

Diversity indices (Shannon-Wiener and Pielou's evenness index) of each sample were calculated by using the R (Ihaka and Gentleman, 1996) package vegan (Oksanen et al., 2016). Bray-Curtis distances were used to compare community structures with non-metric multidimensional scaling (NMDS). The significance of observed differences in community structure was determined by permutational multivariate analysis of variance (PERMANOVA), which is based on Bray-Curtis distance using 9999 permutations. Similarity percentage analysis (SIMPER) was used to identify which OTUs contribute the most for observable difference between groups. Moreover, NMDS, PERMANOVA and SIMPER were carried out using R package vegan. The non-parametric Mann-Whitney (two groups) and Kruskal-Wallis tests (more groups) were employed to evaluate differences. These tests were performed by using SPSS software version 24 (IBM, USA). R pheatmap package (Kolde, 2019) was used to construct a heatmap of the top 10 most abundant OTUs. We identified the OTUs that differed significantly ($q < 0.05$) for two time points with White's non-parametric t-test and Storey's multiple testing correction; the Statistical Analyses of Metagenomic Profiles (STAMP) software version 2.1 was used to perform these tests (Parks et al., 2014).

To determine the correlation between ultraplanktonic fungi communities and environmental factors, canonical-correlation analysis (CCA), was performed using R package vegan. The CCA was performed using the relative abundance of reads data for the fungal phyla and all the physico-chemical factors. To identify environmental factors that significantly correlate with ultraplanktonic fungi communities, an alternative CCA model was constructed using the step function in R and Akaike's information criterion (AIC).

Nucleic acid sequence accession numbers

In this study, the raw sequencing data were submitted to the Sequence Read Archive (SRA) of NCBI (National Center for Biotechnology Information) (Accession number SAMN06107068).

Results

Characterization of environmental variables

In this study, we measured 11 physico-chemical factors at the two sampling sites: CJ01 and CJ05 (*Table A1*). No significant differences were found in the physicophysical-chemical factors between the two sampling sites ($p > 0.3$, Mann-Whitney U-test). Moreover, these factors did not significantly differ across the four time

points (all $p > 0.08$, Kruskal-Wallis test). The whole trophic state of Three Gorges Reservoir is mesotrophic, except CJ05 site, which was slightly eutrophic in summer (TLI = 51.4). At the CJ01 and CJ05 sampling sites, the trophic state did not differ significantly (Mann-Whitney U-test, $p = 1.0$); however, the TLI indexes of CJ05 site were greater than that of CJ01 site (except in summer) (*Table A1*).

Overall community composition of ultraplanktonic fungi

In this study, 283,191 reads (most between 390 and 400 bp) were obtained after removing denoising and chimera. After plotting rarefaction curves of OTU richness for each sample, we inferred that total diversity could be recovered from all the samples (*Fig. A1*). We obtained a total of 35,393 high-quality fungal reads, ranging from 1923 to 7444 per sample. All reads were grouped into 192 OTUs by using a cut-off value of 97% sequence identity. Of the 192 OTUs, 61 OTUs belong to fungi. These fungi OTUs ranging from 28 to 46 OTUs per sample (*Table 1*). The other 131 OTUs mainly affiliated to algae and protozoan. Only three and seven fungal OTUs were unique for CJ01 and CJ05 sites, respectively (*Fig. A2*).

Table 1. *Summary of the diversity index of each sample*

Season	Sample sites	Reads	OTUs	Shannon-Wiener	Pielou evenness
Spring	CJ01	1923	32	1.454	0.419
	CJ05	3140	28	1.442	0.428
Summer	CJ01	3826	43	3.165	0.831
	CJ05	6679	46	2.941	0.764
Autumn	CJ01	6529	44	2.397	0.63
	CJ05	7444	41	2.522	0.671
Winter	CJ01	3526	28	2.517	0.755
	CJ05	2326	30	2.281	0.671

OTU: operational taxonomic unit

Fungal reads were assigned to the following five fungal phyla: *Basidiomycota*, *Ascomycota*, *Chytridiomycota*, *Cryptomycota*, and *Blastocladiomycota* (*Fig. 2A*). Furthermore, there was the group fungi_LKM15, which named after the first clone encountered in an environmental DNA survey in Lake Ketelmeer and referred to some undescribed fungi (van Hannen et al., 1999). The classification of 61 fungal OTUs is as follows: nineteen fungal OTUs belonged to *Ascomycota* (27.05% of total fungi reads); eighteen fungal OTUs belonged to *Basidiomycota* (36.69% of the reads); ten fungal OTUs belonged to *Chytridiomycota* (15.81% of the reads); six fungal OTUs belonged to *Cryptomycota* (18.06% of the reads); one fungal OTU belonged to *Blastocladiomycota* (1.51% of the reads); two fungal OTUs belong to LKM15 group (0.35% of the reads); and five fungal OTUs were considered as unknown fungi (0.53% of the reads). The 10 most frequently observed OTUs constituted 74.07% of total fungal reads, these OTUs belong to *Ascomycota*, *Basidiomycota*, *Chytridiomycota*, and *Cryptomycota* (*Fig. 2B*).

By matching sequences with *Ascomycota*, we observed that there was high affinity with four known classes. Most *Ascomycota* reads were affiliated with *Dothideomycetes* (15.69% of the total fungal reads). Meanwhile, *Eurotiomycetes* and *Sordariomycetes* constituted 3.59% and 4.23% of the total fungal reads, respectively. The most common

Basidiomycota classes were as follows: the *Microbotryomycetes* (15.51% of the total fungal reads), *Exobasidiomycetes* (9.08%), and *Agaricomycetes* (8.03%). *Cryptomycota* was mostly composed of incertae sedis taxa. The diversity of most *Chytridiomycota* was represented by *Zygorhizidium* (74.84% of the total *Chytridiomycota* reads).
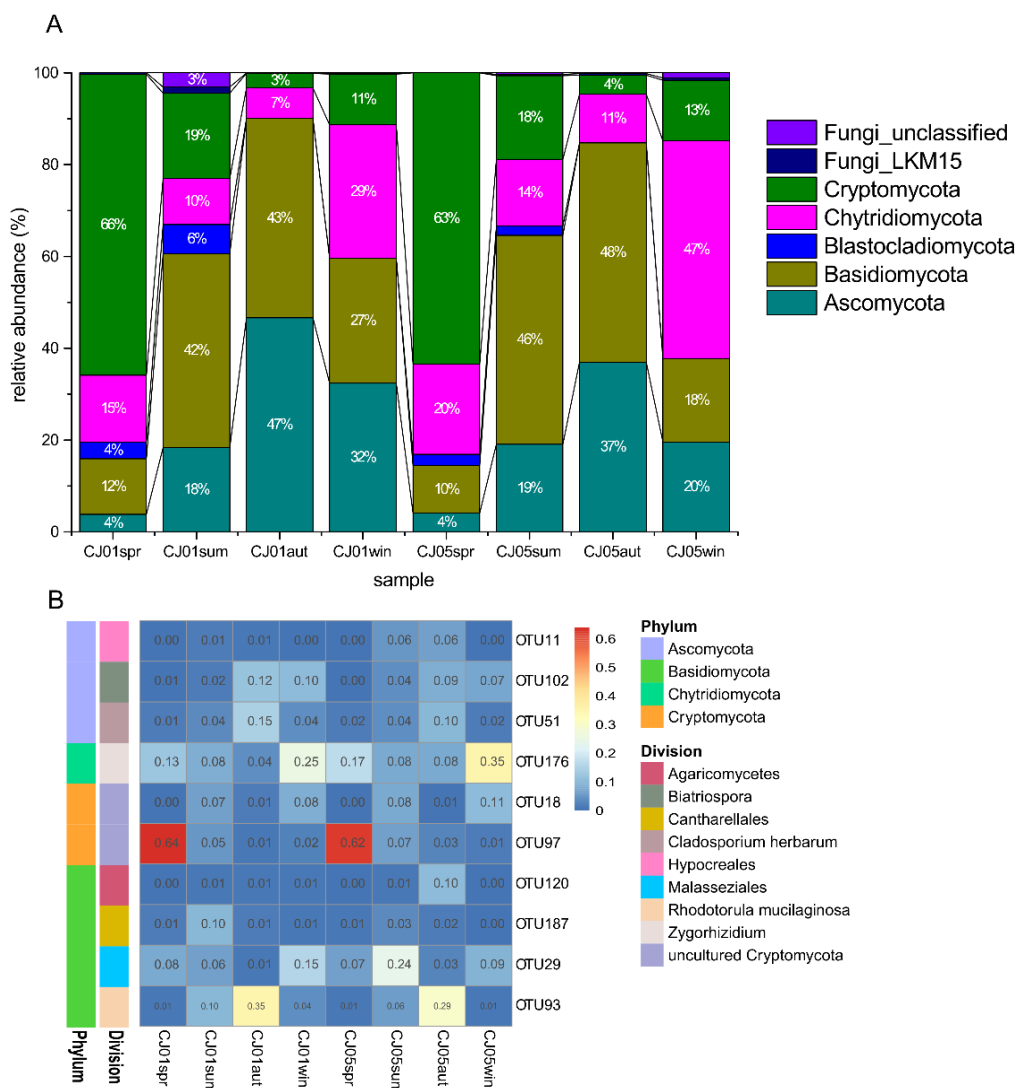

Figure 2. *Variation in fungal community compositions at each sampling site within one year. (A) Taxonomic composition at the phylum level. (B) Heat map showing the relative abundance of the top 10 most abundant operational taxonomic units (OTUs) among the 61 fungal OUTs. Spr: spring, sum: summer, aut: autumn, win: winter*

Only 30 fungal OTUs were assigned to a functional group by FUNGuild database. In this study, six trophic modes and eleven functional guilds were found (*Fig. 3*). Saprotrophs occupied most of the OTUs (14 OTUs); pathotroph-saprotroph possessed most reads (15.52% of total fungal reads) despite containing only one OTU. Saprotroph-symbiotroph occupied one OTU and least reads (2.6% of the total fungal reads). Among the 11 functional guilds, undefined saprotroph had the largest percentage of assigned OTUs (12 OTUs). Plant pathogen (six OTUs) and fungal parasite-undefined

saprotroph (four OTUs) were the other moderately abundant guilds. Animal Endosymbiont-Undefined Saprotrophs comprised of one OTU but most percentages of fungal reads (15.52% of total fungal reads).
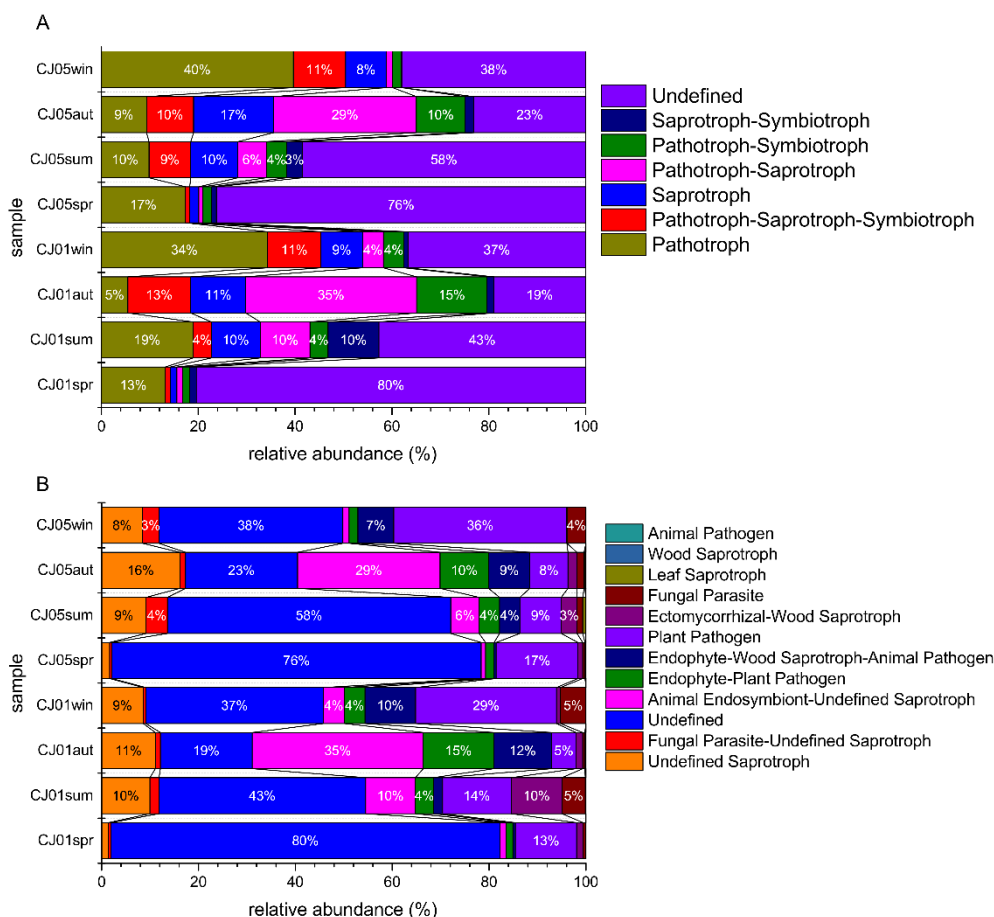

Figure 3. Compositions of fungal functional guild and trophic mode inferred by FUNGuild. (A) Functional guild. (B) Trophic mode

Basidiomycota, *Ascomycota*, and *Chytridiomycota* were assigned to nine, four, and two functional guilds, respectively. *Basidiomycota* was assigned to five trophic modes, while *Ascomycota* was assigned to four trophic modes. The trophic modes of *Chytridiomycota* were pathotroph (two OTUs) and saprotroph (one OTU).

Variations of community structure and composition

The diversity indices (Shannon-Wiener and Pielou's evenness) did not differ significantly between the two sampling sites: CJ01 and CJ05 sites (all $p > 0.8$, Mann-Whitney U-test). Moreover, the diversity indices did not change significantly among the four time points (Kruskal-Wallis test, $p > 0.09$). Fungal communities were primarily differentiated by time (*Fig. 4*) and differences in community structure of ultraplanktonic fungi were significant (PERMANOVA, $p = 0.0101$); no significant difference were found between sampling sites (PERMANOVA, $p = 0.8278$). Based on the overall fungal community composition (at the OTU resolution), SIMPER analysis indicate that

temporal differences were mainly associated with the following OTUs: OTU97 (*Cryptomycota*, 23.94%), OTU93 (*Basidiomycota*, *Rhodotorula*, 15.94%), OTU29 (*Basidiomycota*, *Malasseziales*, 7.196%), OTU51 (*Ascomycota*, *Cladosporium*, 5.94%), OTU102 (*Ascomycota, Biatriospora,* 5.52%) and OTU176 (*Chytridiomycota*, *Zygorhizidium*, 5.28%).
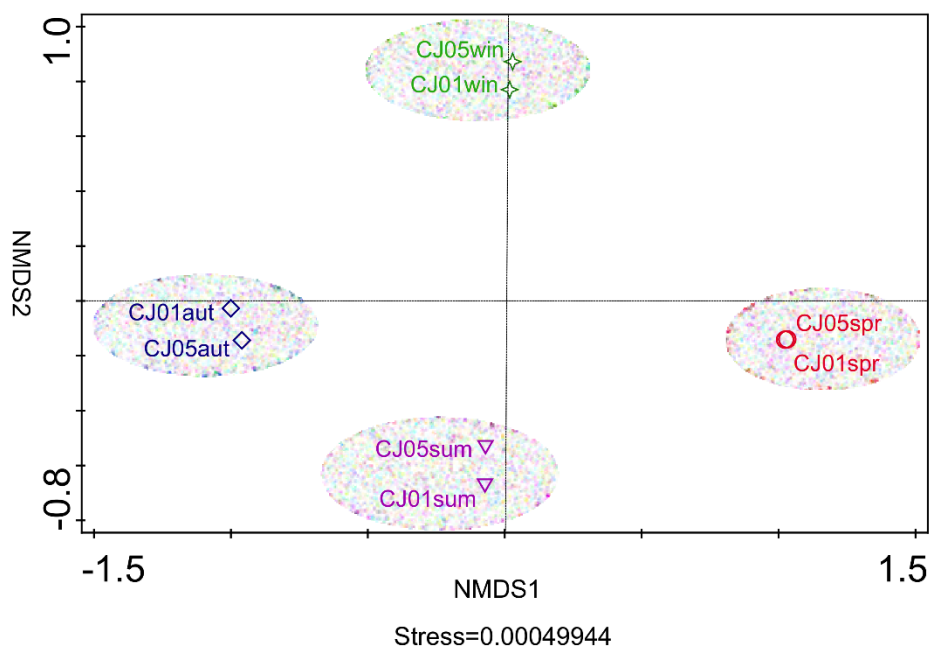

Figure 4. Non-metric multidimensional scaling (NMDS) plot based on Bray-Curtis distances

In this study, dominant phyla varied with time (*Fig. 2A*). *Cryptomycota* and *Chytridiomycota* were dominant in spring and winter seasons, respectively. In warm seasons (summer and autumn), *Basidiomycota* and *Ascomycota* dominated total fungal reads. Most of the top 10 abundant OTUs showed temporal variations (*Fig. 2B*). Seven of the top 10 abundant OTUs achieved maximum abundance in the warmer season, while the other OTUs showed maximum abundance in winter or spring season. For example, OTU176 (*Chytridiomycota*, *Zygorhizidium*) was present in all seasons; however, the abundance of OTU176 was maximum in winter. Moreover, OTU93 (*Basidiomycota*, *Rhodotorula*) was predominant in autumn. Furthermore, OTU97 (*Cryptomycota*) accounted for over 60% of the fungal reads in spring. We found that the relative abundance of three of the 61 OTUs varied significantly between two time points (*Fig. 5*). Compared to summer, the relative abundance of OTU97 (*Cryptomycota*) was significantly higher in spring (White's non-parametric t-test, $q < 0.01$). Moreover, OTU86 (*Ascomycota*, *Trematosphaeria*) was more abundant in autumn than in summer (White's non-parametric t-test, $q < 0.01$). Furthermore, OTU145 (*Basidiomycota*, *Phallus*) was far more abundant in autumn than in winter (White's non-parametric t-test, $q < 0.01$).

Individual OTUs showed variable patterns of temporal change on a finer scale (*Fig. 6*). The read numbers of most OTUs were more abundant in a particular season. Temporal patterns were observed in the relative abundance of these OTUs. Few OTUs showed consistent abundance, regardless of season (for example, OTU 176,

Chytridiomycota). Some OTUs had a relatively low abundance in most seasons; however, they showed a relatively high relative abundance in a certain season (OTU 93, *Basidiomycota*, showed relatively higher abundance in autumn; OTU 97 and OTU 189, *Cryptomycota*, showed relatively higher abundance in winter) or in some seasons (OTU 18, *Basidiomycota* were relatively abundant in following seasons: summer and winter). Furthermore, several OTUs were found consistently in a certain season (OTU 86, *Ascomycota* was found consistently in autumn).

By performing a comparative analysis of the defined functional guild structures in the four time points, we observed a distinct distribution. In the CJ01 site, endophyte-plant pathogen accounted for 15% and 1.46% of fungal reads in autumn and spring, respectively (*Fig. 3*). The trophic mode of pathotroph-saprotroph peaked to 35% in autumn, but it was very low in spring. The percentage of some defined functional guilds, such as undefined saprotroph, was relatively higher in autumn. (11% and 16% in CJ01 and CJ05 sampling sites, respectively) (*Fig. 3A*).
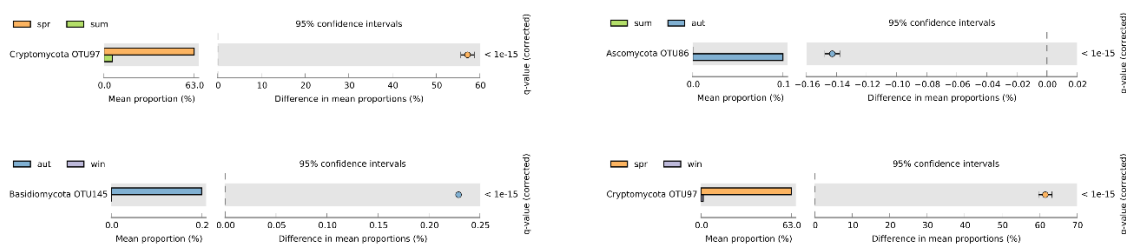

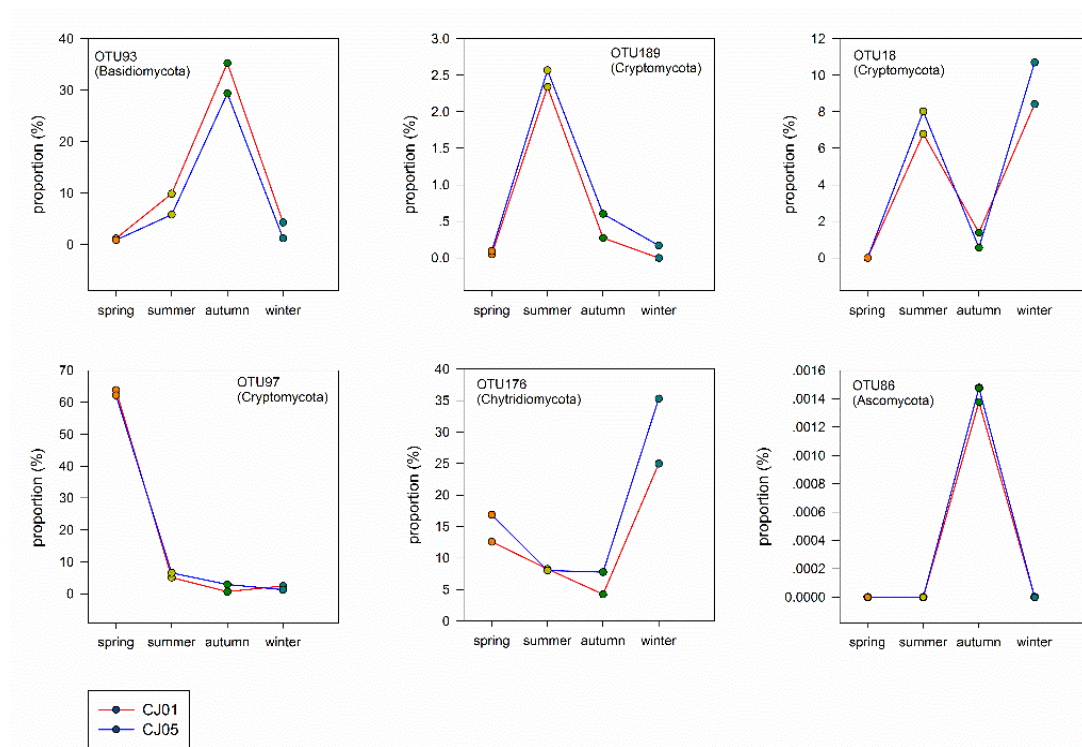**Figure 5.** *OTUs that were significantly different between two seasons*

Figure 6. *Relative abundances of six fungal OTUs showing different types of dynamics*

Correlation of ultraplanktonic fungi and physico-chemical parameters

CCA analysis was conducted for five phyla and three (from 11) forward selected environmental variables (WT, pH, and PO_4^{3-}) (*Fig. 7*). Environmental variables accounted for 91.42% of the variance associated with ultraplanktonic fungi community structure. The first canonical axis accounted for 77.67% of the total variance explained in fungal community structure and were mostly correlated with WT and PO_4^{3-}, while the second canonical axis accounted for 15.35% of the variance and was mostly correlated with pH. The CCA biplot showed that *Basidiomycota* and *Ascomycota* were more related with high temperature. While *Chytridiomycota* and *Cryptomycota* with higher levels of PO_4^{3-}.
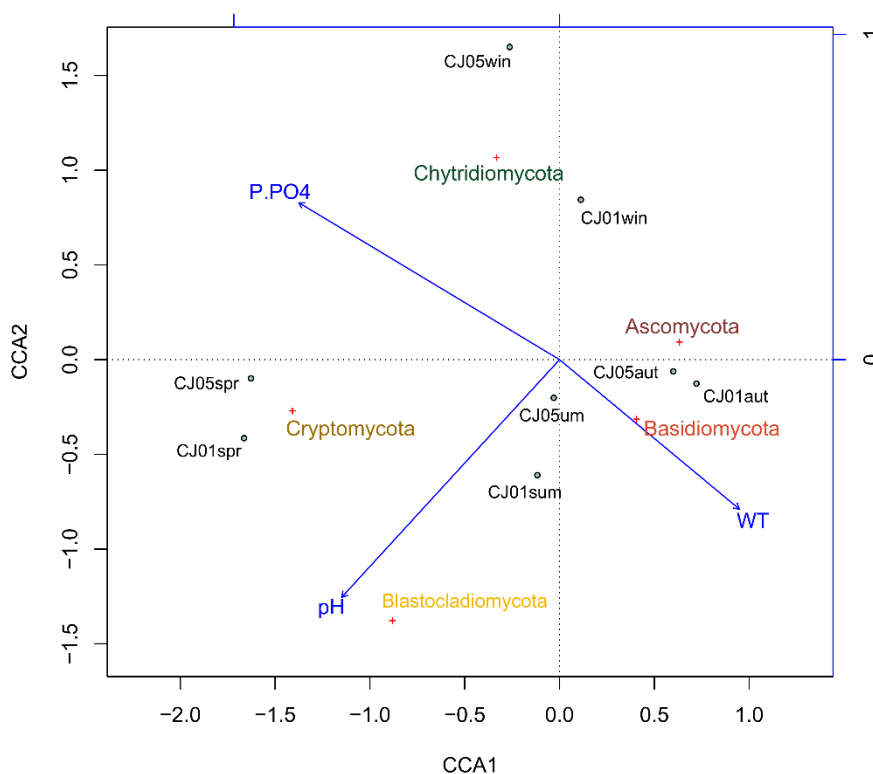

Figure 7. Canonical correspondence analysis (CCA) ordination plot of fungal phylum and environmental parameters. WT: water temperature; P-PO4: orthophosphate

Discussion

It is well known that various commonly used primers used to study microbial diversity of environmental samples may introduce biases during amplification (Tedersoo et al., 2015). Some researchers prefer the SSU rDNA sequence and others prefer ITS regions to survey fungi from environmental samples (Richards et al., 2012). The ITS approach is useful for determining species diversity, but it is of limited use for extrapolating early diverging fungal lineages and investigating novel groups (Horton and Bnms, 2001), therefore ruling out its use as the only marker for phylogenetic studies (Frenken et al., 2017). For early diverging fungal lineages, sequences from the small subunit (SSU) rRNA gene (18S) can provide the affiliation of higher taxonomic ranks (Cole et al., 2014). Some researchers have focused on sampling the SSU rRNA

gene to identify novel fungal diversity among higher taxonomic groups (Jebaraj and Raghulnimar, 2009; Preston, et al., 2012; Kivlin and Treseder, 2015), despite that this gene cannot discriminate between closely related fungal species. In some studies, the small subunit has been particularly useful for phylogenetic placing of *Chytridiomycota* and *Cryptomycota* and other aquatic fungi (Ishii et al., 2015; Panzer et al., 2015). We pay more attention to the new fungal diversity among higher taxonomic groups, so we prefer the SSU rDNA sequence primers.

The significance of planktonic fungi in the large reservoir is unclear, mainly because data on fungal taxonomy and function in this habitat have been limited. Taxonomically, aquatic hyphomycetes that typically decompose leaf litter and wood are mainly associated with Ascomycota, and only a small percentage are affiliated with *Basidiomycota*. In this study, *Ascomycota* and *Basidiomycota* were the dominant phyla in the warm season, but the relative abundance of these phyla was low in the spring (*Fig. 2A*). This change may reflect changes in the function of the whole fungal community. For instance, *Biatriospora* (*Ascomycota*), *Cladosporium* (*Ascomycota*) and *Rhodotorula* (*Basidiomycota*) reach their maximum relative abundance in autumn. *Biatriospora* is associated with plant material found in aquatic habitats (Kolařík et al., 2017). *Cladosporium* species are agents of decay, deterioration, or a cause of allergies or even plant or animal disease, and often have a high environmental impact (Schubert et al., 2007). *Rhodotorula* is a saprophytic fungus that can decompose many substances (Wang et al., 2018).

In this study, the relative abundance of *Chytridiomycota* was the lowest in spring (*Fig. 2A*). In general, the number of parasitic chytrids increases with the growth of phytoplankton (Ibelings et al., 2004). Compared to winter, the relative abundance of *Chytridiomycota* was still much lower in spring (*Fig. 2A*). This phenomenon may be attributed to the dominance of *Cryptomycota* in spring (more than 60%). The dominance of *Cryptomycota* was mainly due to OTU97 (LKM11). Environmental clades LKM11 and *Rozella* formed the deepest-branching clade of fungi (Lara et al., 2010). *Cryptomycota* are parasitic fungi, and some *Cryptomycota* are parasitic in chytrids (Gleason et al., 2014). We hypothesize that OTU97 may be a hyperparasite. Furthermore, the abundance of chytrids may be limited by a large number of *Cryptomycota*. This conforms to the theoretical assumption that both a decline in phytoplankton populations and an increase in hyperparasite populations as the growing season progresses would result in a decrease in chytrid populations (Gleason et al., 2014). Another possible explanation for the dominance of *Cryptomycota* in the spring is that some *Cryptomycota* can parasitize green algae (Held, 1981). In the Three Gorges Reservoir, green algae dominate in spring and reach maximum biomass (Wang et al., 2015). A large number of green algae provide hosts for *Cryptomycota* so that *Cryptomycota* can thrive.

In winter, diatom is the dominant group in the Three Gorges Reservoir, its biomass accounts for the highest proportion (Wang et al., 2015). In the Three Gorges Reservoir, the average water temperature is higher than that of air temperature by 10 ℃ in winter (Zhou et al., 2019). This higher temperature is conducive to algae growth. In this survey, the relative abundance of *Chytridiomycota* was the highest in winter. Generally, the dominance of parasitic fungi in winter is unexpected, given the usual lack of suitable algal hosts during this period. Diatoms reach their maximum biomass in winter could explain the dominance of *Chytridiomycota* with parasitic lifestyles, diatoms provides suitable algal hosts for chytrids. In this study, most taxa of *Chytridiomycota* belong to

the parasitic genus *Zygorhizidium* (80.01% of total *Chytridiomycota* reads). *Zygorhizidium* genus is conducive to the growth of cladoceran zooplankton (Kagami et al., 2007). Thus, chytrid acts as a direct link between inedible or poorly nutritious phytoplankton and filter-feeding zooplankton (Wurzbacher et al., 2010; Haraldsson et al., 2018). This indicates the ecological importance of mycoloop in freshwater ecosystems.

Terrestrial filamentous fungi can be introduced into lakes through spores and pieces of mycelia during inflowing stream, rainwater and wind events (Voronin, 2014). The vegetation on both sides of the Three Gorges Reservoir is dense, and a large number of wood and leaf litter enter the reservoir via flowing streams in the warm season. This litter carries a large number of fungi. In our survey, we found that 18 OTUs only occur in warm seasons. In the *Basidiomycota* and *Ascomycota* phyla, terrestrial taxa are the most dominant taxa. For example, OTU187 (*Cantharellales*) and OTU51 (*Cladosporium*) are considered terrestrial taxa (El-Nagdy and Nasser, 2000). The relative abundance of these terrestrial fungi varies according to time. This variance may be one of the reasons for the temporal variations in the fungal community. Based on our observations, it can be implied that allochthonous inputs are an important source of fungal species in aquatic ecosystems.

In this survey, we also observed an obvious temporal variation in functional guilds of fungi (*Fig. 3*). In cold seasons (spring and winter), fungal parasites dominated functional guilds. With an increase in temperature, the proportion of saprotrophic functional guilds also increases. The highest proportion of saprotrophic functional guilds occurs in autumn. There is tremendous leaf fall in autumn, and a lot of organic matter is input into the reservoir. Saprotrophic fungi depend strongly on the organic carbon content across biomes (Fierer et al., 2009). The input of allochthonous organic matter can impact the fungal community in the reservoir. Therefore, saprotrophic fungi increase as a result of increased organic matter inputs. Seasonal allochthonous inputs are likely important drivers of the fungal community in the reservoir. Interestingly, phytoplankton biomass was highest in summer and lower in autumn in the Three Gorges Reservoir (Wang et al., 2015). The increase in pathotroph-saprotroph OTUs in summer and autumn may be related to the presence and senescence of algal taxa during phytoplankton succession along the season (Sun et al., 2017). However, in this study, only 30 OTUs (30/61, 49.2%) were annotated by the FUNGuild annotated database. The FUNGuild database has been cited by a large number of studies (Lu et al., 2018; Toju et al., 2018; Philpott et al., 2018; Jacobsen, et al., 2018). A previous study revealed that fungal guild characteristics were well represented and that guild assignment relies heavily on accurate OTU taxonomic identification (Nguyen et al., 2016). There are a large number of unassigned groups of fungi in our results, and more accurate results need to be further studied.

Chytridiomycota and *Cryptomycota* were mostly related with higher PO_4^{3-} levels. Fungi react indirectly to physico-chemical factors; this implies that PO_4^{3-} ions may be indirectly affecting these fungi. Furthermore, PO_4^{3-} ion is a limiting nutrient in aquatic systems, and it is especially critical for autotrophs (McMahon and Read, 2013). The presence of phosphorus in lakes is positively correlated with phytoplankton (Lv et al., 2011). In this study, some of the recovered *Cryptomycota* and *Chytridiomycota* OTUs (e.g. OTU125, *Rhizophydiales*) are parasites of phytoplankton. These parasites extract nutrients from phytoplankton (Arce Funck et al., 2015). So, plankton can be beneficial for the growth of fungi. Therefore, PO_4^{3-} levels can influence the abundance of fungi. In

our investigation, we found that the diversity of fungi is reduced by eutrophication. This finding has also been reported by researchers in previous studies (Duarte et al., 2015).

Conclusion

To conclude, our study showed that the Three Gorges Reservoir region harbors high-level fungal diversity with an obvious temporal variation of community composition and functional guilds. Parasites thrive in the spring and winter and saprotrophs prevail in the autumn. This highlights a temporal change in the dominant functional role of fungi in the reservoir. Our results show that terrestrial inputs may be an important source of fungal species in reservoir ecosystems. A high species richness and many aquatic hyphomycetes were highlighted, suggesting important ecological roles for fungi in the reservoir region. Finally, to obtain insight into the activity of aquatic fungi and their associated functions, more effective approaches will be necessary in future studies.

Competing interests. The authors declare no competing financial interests.

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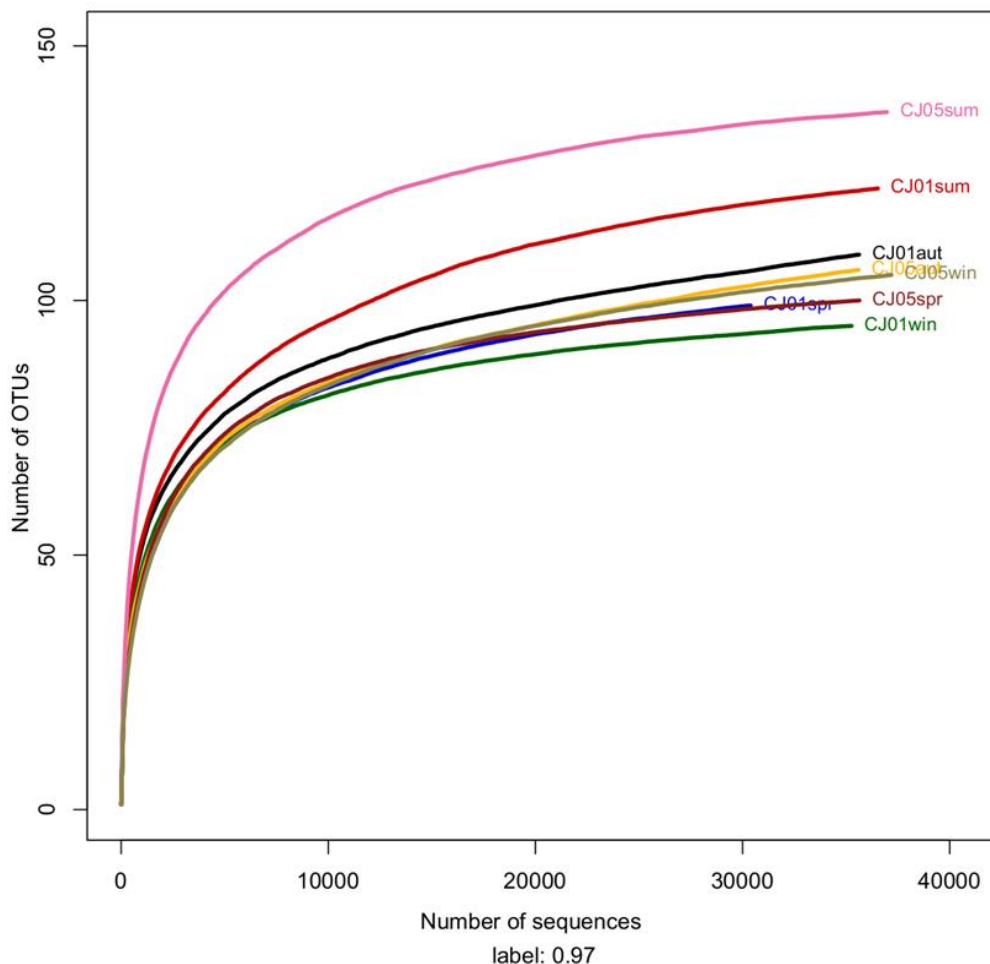
APPENDIX

Figure A1. Rarefaction curves. (Spr: spring, sum: summer, aut: autumn, win: winter)
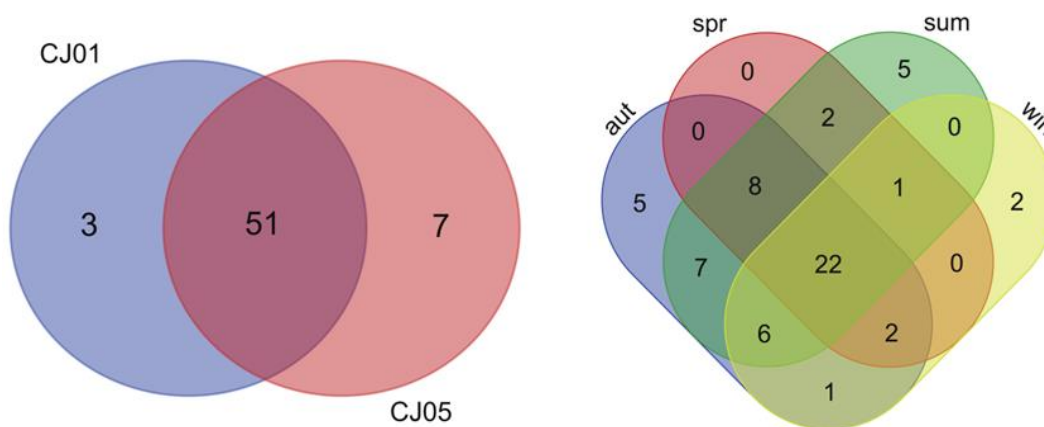

Figure A2. Venn diagrams showing shared and unique seasons and sampling sites by operational taxonomic unit (OTU)

Table A1. *Physical and environmental characteristics of the sampling sites*

	CJ01spr	CJ01sum	CJ01aut	CJ01win	CJ05spr	CJ05sum	CJ05aut	CJ05win
TP (g/L)	0.075	0.084	0.079	0.074	0.079	0.035	0.139	0.077
TN (mg/L)	1.911	2.006	2.122	1.800	1.889	2.880	2.255	2.033
COD (mg/L)	1.143	1.451	2.222	1.569	1.219	1.725	2.343	2.000
P-PO4 (mg/L)	0.076	0.054	0.051	0.064	0.076	0.050	0.054	0.075
N-NH4 (mg/L)	0.027	0.026	0.069	0.094	0.006	0.039	0.028	0.072
N-NO3 (mg/L)	1.667	1.378	1.149	1.718	1.635	1.327	1.184	1.787
WT (℃)	14.800	26.820	22.280	18.440	15.000	26.570	22.210	18.410
DO (mg/L)	9.130	6.730	7.530	9.120	10.050	6.720	7.850	9.370
ORP (mV)	332.400	127.000	186.000	247.000	335.500	83.000	195.000	258.000
pH	8.750	8.470	8.020	8.160	8.700	8.460	8.050	7.490
Turbidity (NTU)	1.200	4.500	2.100	1.500	1.400	5.800	1.900	1.400
TLI	40.692	43.703	47.433	43.144	41.488	42.429	51.414	46.178

Spr: spring, sum: summer, aut: autumn, win: winter. WT: water temperature, DO: dissolved oxygen, TN: total nitrogen, P-PO4: orthophosphate, N-NO3: nitrate, TP: total phosphorus, N-NH4: ammonium, COD: chemical oxygen demand, ORP: oxidation-reduction potential, TLI: trophic level index

ELECTRONIC APPENDIX

This manuscript has five electronic appendices with basic data:

Appendix 1. Information on the classes and orders identified from the 8 samples
Appendix 2. Compositions of fungal functional guild inferred by FUNGuild
Appendix 3. The distribution of fungal functional guild in the phylum level
Appendix 4. The distribution of fungal trophic mode in the phylum level
Appendix 5. Similarity Percentage analysis (SIMPER) of the 61 fungal OTUs