Deciphering Linkages Between Microbial Communities and Priming Effects in Lake Sediments With Different Salinity

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Abstract Priming effects (PEs) and their associated microbial drivers are not well studied in lake sediments. Here, we investigated PEs and underlying potential microbial drivers in the sediments of lakes on the Qinghai-Tibetan Plateau (QTP). Sediments were collected from three QTP lakes with different salinity, followed by microcosm construction and subsequent incubation at in situ temperature. The sediment microcosms were amended with ¹³C-labeled glucose, on which PE intensities were evaluated in the incubations on Days 7 and 42. Positive PEs were observed in all the studied lake sediment microcosms. PE intensities exhibited significantly (p < 0.05) linear correlations with most of the measured physicochemical factors (e.g., salinity, sediment total nitrogen/phosphorus, and ratios of carbon:nitrogen), and such linear correlations were inverse for the early (i.e., on Day 7) and late (i.e., on Day 42) PEs. Prokaryotic and fungal community compositions significantly changed owing to glucose addition in the studied lake microcosms, suggesting that both prokaryotes and fungi may contribute to the observed PEs. Network analysis showed that the numbers of positive correlations between fungal taxa and other microorganisms increased with the enhancement of the late PE intensity, suggesting that fungi and associated co-metabolisms may play key roles in late PEs in this study. Collectively, this study gives new insights into PE intensity and underlying microbial drivers of PE in lake sediments, and such knowledge is of great importance to understanding organic matter mineralization in lake ecosystems.

1. Introduction

Priming effect (PE) is an increase or decrease (termed as positive and negative PE, respectively) in organic carbon mineralization in response to inputs of fresh organic matters (OMs) (Guenet et al., 2010; Kuzyakov, 2010). Microorganisms (including archaea, bacteria, and fungi) play crucial roles in driving PE-generation processes (Fontaine et al., 2011; Morrissey et al., 2017). It is generally accepted that positive PEs can be generated through two distinct microbial processes: stoichiometric decomposition and nutrient mining (Chen et al., 2014; Fontaine et al., 2004; Razanamalala et al., 2018). In the stoichiometric decomposition process, organic carbon mineralization is increased by collateral damage of redundant extracellular enzymes released by microbial decomposers in response to OM input, whereas in the nutrient mining process, organic carbon mineralization is enhanced by co-metabolisms of two types of co-occurring microbial populations (Blagodatskaya & Kuzyakov, 2008; Fontaine et al., 2003; Razanamalala et al., 2018). Two co-metabolism types are proposed to drive positive PEs: (1) in the presence of fresh OM, one microbial population can degrade extant recalcitrant OM resulting in intermediate products that are not usable for themselves but mineralizable for another microbial population; and (2) one microbial population utilizes fresh OM, resulting in catabolites that stimulate the growth of another microbial population capable of mining native OM as nutrients (e.g., nitrogen) (Guenet et al., 2010). Through the two above-mentioned co-metabolisms, the recalcitrant or native OM is cooperatively degraded by two co-occurring microbial populations during the positive PE generation process, and the relative abundances of these two microbial populations are likely positively correlated with each other among samples. Up to now, microbial mechanisms for PEs have been intensively investigated in numerous studies, and a broad range of microbial populations were shown to
drive positive PEs. For instance, several previous studies indicated that various bacterial (e.g., *Proteobacteria*) and fungal (e.g., *Ascomycota*) groups were potentially involved in positive PEs (Fan et al., 2019; Fang et al., 2018; Fontaine et al., 2011; Morrissey et al., 2017; Pascault et al., 2013; Razanamalala et al., 2018). However, it is poorly known which microbial populations engage in co-metabolism and drive positive PEs.

Several studies pointed out that both stoichiometric decomposition and nutrient mining processes can co-occur in one sample, and they are regulated by environmental factors (e.g., nutrient status and temperature) during positive PEs in soil ecosystems (Chen et al., 2014; Razanamalala et al., 2018). For example, the former process is favored at the condition of rich nutrients or low temperature, while the latter prefers the condition of nutrient limitation or high temperature (Chen et al., 2014; Razanamalala et al., 2017). However, little is known whether salinity, one type of environmental determinant, can affect PE generation processes. Salinity is a key environmental factor influencing microbial activity and community composition in natural environments (Lozupone & Knight, 2007; Oren, 2011; Yang et al., 2019). For example, sediment microbial activity (e.g., CO$_2$ production and enzyme activity) decreases with increasing salinity (Reed & Martiny, 2013); microbial community compositions are more dissimilar among samples with larger salinity difference (Cottrell & David, 2003; Herlemann et al., 2011; Jiang et al., 2007; Wu et al., 2006; Yang et al., 2016). As a consequence, it can be speculated that salinity may affect PE intensity by selecting for certain microbial members or limiting microbial enzyme activity. However, it has been poorly known to date whether salinity could affect PE intensity and what microbial populations drive PEs at different salinity.

Positive PEs have been frequently reported in soil ecosystems (Fang et al., 2018; Liu et al., 2017; Perveen et al., 2019; Razanamalala et al., 2018). By contrast, a few documents reported on the occurrence of positive PEs and associated generating mechanisms in lake sediments (Bengtsson et al., 2018; Guenet et al., 2014), which are globally distributed active sites for mineralization of considerable amounts of autochthonous and allochthonous OMs (Cole et al., 2007; Gudasz et al., 2010; Tranvik et al., 2009). Therefore, it is of great importance to unveil PE generation and associated mechanisms in lake sediments. Three potential ways have been proposed to stimulate OM mineralization through positive PEs in aquatic ecosystems: (1) submerged vegetation irregularly release metabolites (e.g., sugars) into sediments, which further stimulate microbial mineralization of native OM; (2) phytoplankton blooms pulse fresh OM, inducing microorganisms to mineralize old sediment OM; and (3) inputs of terrestrially derived OM (e.g., leaf and grass litters) cause additional sediment OM mineralization (Bengtsson et al., 2018). Although positive PEs have been suggested to be able to occur in lake sediments (Guenet et al., 2014), direct evidences still lack. Furthermore, a previous study indicated that an average PE intensity was 23.7% of basal respiration in lake waters; however, little is known about PE intensity and their microbial drivers in lake sediments (Bengtsson et al., 2018).

To fill the above knowledge gaps, this study investigated PEs and associated microbial drivers in the sediments from three Qinghai-Tibetan lakes with different salinity through microcosm experiments followed by geochemical and microbial analyses. The major objectives of this study were to (1) investigate PE intensities and their microbial drivers in the studied lake sediments, (2) discern the key microbial taxa potentially performing co-metabolisms during PEs, and (3) explore the influence of salinity on PE-generation processes among the studied lake sediments.

2. Materials and Methods

2.1. Field Sampling

Sampling cruise was conducted in June 2018. Three Qinghai-Tibetan lakes with different salinity were chosen for this study: Erhai Lake (EHL) is a freshwater lake; Qinghai Lake (QHL) and Gahai Lake (GHL) are two saline lakes (Yang et al., 2013). The salinity and pH of the sampling sites were measured with portable meters (SANXIN, Shanghai, China), and the portable meters were calibrated according to manufacturer instruction before the field sampling. In the field, about 2-L surface water (0–10 cm) of each lake was collected into sterilized 2-L polycarbonate bottles (Nalgene, USA) for laboratory incubations. Surface lake water was filtered through combusted Whatman GF/F filters with 0.7-μm pore size for measurements of dissolved organic carbon (DOC), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP). The DOC samples were collected into dark glass vials preciliated with concentrated phosphoric acid (~100 μL). Surface sediments (approximately 0–5 cm) were sampled at the inshore sites (~5–10 m away from shoreline, ~1-m water depth) of each lake using a grab-bucket collection sampler, and approximately 2 kg...
of sampled sediments were collected into sterilized plastic zip bags. All the collected samples were kept at 4°C and in the dark in the field and during transportation. Samples were immediately processed on arrival in laboratory.

### 2.2. Laboratory Analyses

Sediment TOC samples were acidified with 1 M HCl to remove carbonates, rinsed to neutral pH with deionized water, dried at 60°C, and finally ground to powder. DOC and TDN concentrations were analyzed on a multi N/C 2100S analyzer (Analytik Jena, Germany). Sediment TOC and total nitrogen (TN) contents were analyzed using a 2400 Series II CHNS/O Analyzer (Perkin Elmer, Waltham, MA, USA). TDP and sediment total phosphorus (TP) were analyzed colorimetrically using a published phosphomolybdic acid method (Neal et al., 2000). Before TP measurement, sediment was firstly treated with perchloric acid (~12 mol L⁻¹), and then the resulting extracts were used to analyze TP contents.

### 2.3. Experiment Setup

#### 2.3.1. Preparation of ¹³C-Labeled Glucose

According to many previous studies in soils (Guenet et al., 2014; Mau et al., 2015; Morrissey et al., 2017; Razanamalala et al., 2018), glucose was selected for our microcosm experiments. Note that glucose is unusual in lake waters, but it is likely present in root exudation of submerged vegetation in lakes (Zhang et al., 2017). A uniformly ¹³C-labeled glucose (≥99 atom % ¹³C, Sigma-Aldrich, USA) was used in the experiment, and its initial δ¹³C value was 8,809.023‰. To minimize technical constraints of the isotope ratio mass spectrometer, the initial ¹³C-labeled glucose chemical was diluted with nonlabeled glucose (Sigma-Aldrich, USA). The final δ¹³C value of the diluted glucose was 1,201‰ as measured by a MAT 253 isotope ratio mass spectrometer (Finnigan MAT, Germany). δ¹³C value was calculated based on the following equation: 

\[
\delta^{13}C = \left( \frac{R_{\text{sample}} - R_{\text{standard}}} {R_{\text{standard}}} - 1 \right) \times 1,000
\]

where \( R_{\text{sample}} \) and \( R_{\text{standard}} \) are the ratios of ¹³C and ¹²C isotopes in the sample and standard, respectively. \( R_{\text{standard}} \) (PDB) is 0.011180.

#### 2.3.2. Microcosms Incubation and Sample Collection

The sampled sediments were mixed (water:sediment = 1:1, v:v) with the sampled water from each lake. The resulting sediment slurries were employed to construct six microcosms (i.e., three treatments and three controls) for each lake. These microcosms were established by distributing ~200 g sediment slurries into ~1.1-L sterilized glass bottles. Prior to experimental incubation, the constructed microcosms were preincubated at 15°C (similar to in-situ temperature) in the dark for 3 days. Subsequently, the three microcosm treatments were amended with 0.02-g diluted glucose (as prepared before) to reach a final glucose concentration of 100 mg L⁻¹. This glucose concentration is possibly higher than most of known aquatic ecosystems but is a classical input for the PE experiments in soils (Guenet et al., 2014). The microcosm controls were not amended with glucose. Before incubation, approximately 0.5-g sediment was collected from each of the microcosm treatments as initial samples for DNA analysis.

All the microcosm treatments and controls were incubated aerobically in the dark at 15°C for 42 days and sealed with air permeable and microbe proofing films. To avoid the effect of water evaporation, all microcosms were supplemented with their corresponding lake waters (stored at 4°C) every week. For carbon dioxide (CO₂) concentration and carbon isotope ratio analyses, all incubated bottles (including treatments and controls) were first flushed with fresh air and then sealed with rubber plugs. Subsequently, the gas phase was allowed to accumulate for 24 hr. Gas samples were collected from the headspace of the incubated bottles using a 50-ml injection syringe. Meanwhile, ~0.5-g sediment was taken in duplicate (to minimize random process effect) from each of the microcosms for DNA samples (Zhou et al., 2013). After gas sampling, all the microcosms were still incubated under aerobic conditions. The gas samples were immediately analyzed after they were retrieved, and DNA samples were stored at ~80°C for further DNA extraction. We only collected gas and DNA samples on Days 7 and 42 respectively, which correspond to early and late stage of PE (Razanamalala et al., 2017).

### 2.4. CO₂ and δ¹³C Measurements

The total CO₂ concentration of the gas samples were analyzed by a gas chromatography (Agilent GC7890A, USA) equipped with a thermal conductivity detector. Briefly, the oven, injector and detector temperatures of the gas chromatography were set as 60°C, 100°C, and 200°C, respectively. Standard curves were constructed by using serial dilutions (10⁻⁴ ppm) of standard CO₂ gases with Pearson correlation
coefficients of $R^2 > 0.998$. The CO$_2$ concentrations were calculated on the basis of the established standard curves. The detection limit and precision of the gas chromatography are 10 ppm and <1%, respectively. For carbon isotope analysis, the gas samples were firstly purified at 25°C using an off-line vacuum system, and then carbon isotope ratios of the CO$_2$ in the studied gas samples were measured using a Finnigan MAT 251 isotope ratio mass spectrometer with dual-inlet system (Finnigan MAT, Germany). The accuracy of carbon isotope analysis was controlled by standard CO$_2$ cylinder gas, and standard deviation was less than 0.05‰. PE evaluation followed a previously published method (Mau et al., 2015; Morrissey et al., 2017).

Briefly, priming intensity was calculated based on the difference between CO$_2$-C derived from sediment organic carbon (SOC) mineralization with glucose addition and the CO$_2$-C derived from SOC mineralization without glucose addition as follows: PE intensity = $C_{\text{treatment}} \times (\delta_{\text{treatment}} - \delta_{\text{glucose}}) / (\delta_{\text{control}} - \delta_{\text{glucose}}) - C_{\text{control}}$, in which $C_{\text{treatment}}$ and $C_{\text{control}}$ (i.e., basal respiration) indicate average CO$_2$-C values from microcosm treatments and controls (reported as mg C g SOC day$^{-1}$), respectively. $\delta_{\text{glucose}}$, $\delta_{\text{treatment}}$, and $\delta_{\text{control}}$ represent the $\delta^{13}$C values of the diluted glucose, of the CO$_2$ in the microcosm treatment and control headspaces, respectively.

### 2.5. DNA Extraction and Quantitative PCR

Total DNA was extracted from the sampling sediments (~0.5 g) by using the Fast DNA SPIN Kit for Soil (MP Biomedical, USA) following the manufacture procedures. The prokaryotic 16S rRNA and fungal internal transcribed spacer (ITS) gene abundances in the studied sediment samples were quantified by quantitative polymerase chain reaction (qPCR) with the use of primer sets: 515F (5′-GTGYCAGCMGCCGCGGTAA-3′)/806R (5′-GGACTACNVGGGTWTCTAAT-3′) and ITS1f (5′-CTTGGTCAATTAGAGGAAATGA-3′)/ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′), respectively (Walters et al., 2015). qPCRs were conducted in triplicate on an ABI 7500 real-time PCR system (Applied Biosystems, Carlsbad, CA, USA) with a reaction volume of 20 μl, containing 10 μl of 2 × SYBR® Premix Ex TaqTM (Takara, Japan), 0.4 μM of each primer, 0.4 μl of ROX Reference Dye II (50×), and 2 μl of DNA template. Standard curves were constructed by using serial dilutions ($10^2$–$10^5$ copies) of plasmids (pGEM-T) containing cloned 16S rRNA and ITS genes with Pearson correlation coefficients of $R^2 > 0.99$. PCR efficiencies were 90–100%. The quality and length of the qPCR products were checked by dissociation curve analysis and 1% agarose gel electrophoresis. The qPCR results were reported as the number of (partial) gene copies per gram (copies g$^{-1}$) of wet sediment.

### 2.6. Illumina Sequencing

The primer sets of 515F/806R and ITS1f/ITS2 were used for PCR amplifications of the 16S rRNA and ITS genes in the studied samples, respectively, following a previously published method (Walters et al., 2015). Unique 12-bp barcodes were attached between the sequencing adapter and reverse primer to differentiate among samples. Triplicate PCR reactions were carried out for each sample, and the PCR products (~400 bp) were purified using a DNA Gel Extraction Kit (Axygen, USA). The triplicate PCR amplicons from each sample were pooled with equimolar concentrations. To assess sequencing quality, the amplicon libraries were combined with PhiX control V3 library (Illumina) (final conc. at 20%), followed by sequencing with an Illumina Hiseq 2500 platform (paired-ends sequencing of 2 × 250 bp).

Raw 16S rRNA and ITS gene sequences were firstly demultiplexed and adaptor trimmed by using the Illumina real time analysis software (version 1.18.66.3, Illumina, San Diego, CA, USA). Subsequently, those demultiplexed sequences were analyzed by the USEARCH (v.11.0.667) program (http://www.drive5.com/usearch/). The default parameters were applied in the present analyses unless indicated otherwise. Briefly, the functions of fastq_mergepairs, fastx_truncate (parameters: -stripleft 19 and -stripright 20 for 16S rRNA gene sequences; -stripleft 22 and -stripright 20 for ITS sequences), fastq_filter (parameters: -fastq_maxee 1.0, -fastq_minlen 200, -fastq_maxqs 0 and -fastq_truncqual 30), unoise3, and otutab (parameter: -sd 1.0) were conducted in turn to merge pair-ends reads, trim primers, quality filter reads, denoise, and remove chimeras and to generate zero-radius operational taxonomic unit (ZOTU) tables. To minimize the bias for ITS gene analysis, we removed the flanking small ribosomal subunit (SSU) and 5.8S genes from the ITS sequences by using ITSx (v1.0.11) software (http://microbiology.se/software/itsx/) after quality filtering. The taxonomy of the prokaryotic and fungal ZOTU sequences was assigned by using the usearch-sintax function (Edgar, 2016) with an 80% confidence against the databases of the Ribosomal Database Project (RDP) v.11.5 and UNITE v.7.1 (http://unite.ut.ee) for 16S rRNA and ITS genes, respectively. Nonprokaryotic and
nonfungal sequences were culled out from the ZOTU tables. Singletons and ZOTU containing <10 reads were removed from the ZOTU tables. The ZOTU tables were finally rarified to equal sequence number (i.e., the minimum sequence number among the studied samples, \( n = 8,029 \) for the 16S rRNA ZOTU table; \( n = 4,989 \) for the ITS ZOTU table) for each sample before downstream analyses. Alpha diversity indices (e.g., observed ZOTUs, Shannon, and Equitability) and rarefaction curves were calculated with the use of R package “vegan” (Oksanen et al., 2019).

### 2.7. Statistical Analyses

All statistical analyses in the present study were performed using R program (version 4.0.0) with various R packages (R-Core-Team, 2020) unless otherwise indicated. Linear fitting was performed to discern the correlations between PE intensities and physicochemical parameters using the \( \text{lm} \) function of R package “stats.” Nonmetric multidimensional scaling (NMDS) and clustering analyses (unweighted pair group method using arithmetic average) were used to visualize the difference of prokaryotic and fungal community compositions among samples (derived from different lakes and experimental time points) based on the Bray-Curtis dissimilarity using the “vegan” package. To test the difference of prokaryotic and fungal community compositions between treatment and control samples collected from different time points (i.e., Days 7 and 42), analysis of similarities (ANOSIM) was performed based on Bray-Curtis dissimilarity with 9,999 permutations using the R package “vegan.” The \( t \) test function was applied to discern the difference of ZOTU richness and gene abundances between the treatment and control samples using the R package “stats.” To discern the prokaryotic and fungal ZOTUs whose relative abundances showed significant increase due to glucose addition, the Wilcoxon rank sum tests were used to compare the difference of relative abundances between the treatments and controls using the R package “stats.” We manually defined the significantly apparent increasing ZOTUs by whose relative abundances in treatments significantly (\( p < 0.01 \)) different with their corresponding controls and whose average relative abundance in the treatments were higher >1% than their corresponding controls.

In addition, network analysis was applied to discern any potential cooperation relationships between the prokaryotic and fungal species for each lake treatment. Sparse correlations for compositional (SparCC) data were analyzed following a previous description (Friedman & Alm, 2012). The rare ZOTUs (relative abundance <0.01%) in each sample were removed before SparCC analysis. ZOTUs among samples that occurred simultaneously at least eight samples were included in the SparCC analyses. To controlling the false discovery rates, all \( p \) values generated by SparCC analyses were adjusted using the Benjamini-Hochberg method (Benjamini & Hochberg, 1995). SparCC coefficient \( |R| > 0.7 \) with \( p < 0.01 \) was considered as robust correlations in that it gave >50% explanation in linear model fitting (Steinhauser et al., 2008). We assumed that the relative abundances of the prokaryotic and fungal ZOTUs were positively correlated with each other if they cooperatively degrade native OM. Therefore, only positive SparCC correlations were considered as potential relationships of co-metabolisms among the prokaryotic and fungal taxa. All the robust correlations would form correlation networks where nodes represented the prokaryotic and fungal ZOTUs, and edges indicated significantly positive correlations between nodes. The positive networks were visualized on the interactive platform Gephi 0.9.2 (https://gephi.org/).

The average connectivity (indicating a complexity of the network) of each network was calculated by using the R package of igraph (Csardi & Nepusz, 2006). The network modularity and module division were determined by an algorithm of fast greedy optimization (Clauset et al., 2004) using the R package of igraph. Nodes in the network can be classified into different groups (i.e., modules) according to their connectivity, and certain node may be connected with other nodes in their own module or other modules. The within-module connectivity (Zi) and among-module connectivity (Pi) were calculated to assign the roles of network nodes following a previous study (Guimerà & Nunes Amaral, 2005). Four categories can be defined for each node: (1) peripheral nodes (\( Zi < 2.5, Pi < 0.62 \)) are indicated by the nodes with low connectivity, and they are usually connected with nodes in their own module; (2) connectors (\( Zi < 2.5, Pi > 0.62 \)) are indicated by the nodes with high connections to several other modules; (3) module hubs (\( Zi > 2.5, Pi < 0.62 \)) are indicated by the nodes with high connections to multiple nodes in their own modules; and (4) network hubs (\( Zi > 2.5, Pi > 0.62 \)) are indicated by the nodes with high connections with nodes not only in their own modules but also in other modules (Deng et al., 2012; Zhou et al., 2011). The network and module hubs and connectors were generally proposed as keystone members in the network (Deng et al., 2012). Note that the above...
statistical approaches using DNA sequencing data cannot identify the active microbial communities or a real cooperation between microbial taxa in the studied lake microcosms. However, it can at least provide some insights into putative relationships between microbial populations and PE.

3. Results

3.1. Geochemical Conditions

The basic geochemical parameters of the studied lakes were shown in Table 1. Briefly, the salinities of the studied EHL, QHL, and GHL were 1, 16, and 34 g L\(^{-1}\), respectively; pH values of these lakes were 8.9, 9.5, and 9.3, respectively. The water geochemical parameters of the initial microcosm slurries were 17.4–38.9, 3.2–13.9, and 0.1–0.2 mg L\(^{-1}\) for DOC, TDN, and TDP, respectively, while sediment geochemical parameters were 13.8–28.6, 0.5–1.9, and 0.1–0.2 mg g\(^{-1}\) for TOC, TN, and TP, respectively (Table 1). The dry weights of the sediment in each EHL, QHL, and GHL microcosms were 61.2, 33.3, and 65.4 g, respectively.

3.2. PE in the Studied Lake Sediments

Positive PEs were observed on Days 7 and 42 in all the studied lake sediment microcosms and the priming intensities on Day 7 were higher than those on Day 42 (Figure 1). On Day 7, average PE intensities were 7.34 ± 1.10, 0.94 ± 0.11, and 1.42 ± 0.06 mg C g SOC day\(^{-1}\), and average basal respirations were 6.89 ± 0.93, 2.65 ± 0.04, and 4.48 ± 0.07 mg C g SOC day\(^{-1}\) for the EHL, QHL, and GHL lake microcosms, respectively (Table S1). On Day 42, the average PE intensities were 0.40 ± 0.09, 0.67 ± 0.09,
and 1.09 ± 0.09 mg C g SOC day, and average basal respirations were 3.13 ± 0.12, 1.73 ± 0.08, and 1.34 ± 0.05 mg C g SOC day for the EHL, QHL, and GHL lake microcosms, respectively (Table S1). The PE intensity ranged between 12.8% and 106.6% of basal respiration. PE intensities showed a significantly ($p$ < 0.05) positive linear relationship with basal respiration on Day 7, whereas an opposite trend was observed on Day 42 (Figure 1). Furthermore, PE intensities on Days 7 and 42 exhibited significantly positive/negative linear relationships with most of the measured physicochemical parameters including salinity, pH, TDN, TDP, TP, and C:N ratios (Figure S1). However, such linear relationships were inverse between the PEs on Days 7 and 42 (Figure S1).

3.3. Variation of the Prokaryotic 16S rRNA and Fungal ITS Gene Abundance

The prokaryotic 16S rRNA gene abundances were $5.8 \times 10^5$ to $2.0 \times 10^9$ copies g$^{-1}$ among the studied microcosm treatments, and $7.0 \times 10^5$ to $1.3 \times 10^9$ copies g$^{-1}$ among the studied microcosm controls (Figure 2 and Table S2). The fungal ITS gene abundances were $1.6 \times 10^5$ to $3.6 \times 10^6$ copies g$^{-1}$ among the studied microcosm treatments, and $1.8 \times 10^5$ to $2.2 \times 10^6$ copies g$^{-1}$ among the studied microcosm controls (Figure 2 and Table S2). The average prokaryotic 16S rRNA and fungal ITS gene abundances in the microcosm treatments were higher than that in their corresponding controls (Figure 2). Significant ($t$ test: $p$ < 0.05) differences of the prokaryotic 16S rRNA and fungal ITS gene abundances were observed between the microcosm treatments and their corresponding controls in the EHL and QHL microcosms on Day 42 and in the QHL and GHL microcosms on Day 7 (Figure 2).

3.4. Variation of Prokaryotic and Fungal Community

A total of 1,834,051 and 2,439,427 reads were obtained for the prokaryotic 16S rRNA and fungal ITS genes in this study. Rarefaction curves of the studied samples were (nearly) saturated (Figure S2). The observed prokaryotic ZOTUs were 538–1,179 and Shannon indices were 4.5–5.9 among the studied samples (Table S3), while the observed fungal ZOTUs among the studied samples were 113–413 and Shannon indices were 1.5–4.6 (Table S4). The observed prokaryotic or fungal ZOTUs showed significant differences...
between certain treatments and their corresponding controls (Figure S3). For example, the observed prokaryotic ZOTUs were significantly (t-test: p < 0.05) less in the EHL microcosm treatments sampled on Day 42 than that in their corresponding controls, whereas an opposite trend was observed for the QHL microcosm treatments and controls on Day 42 and the observed fungal ZOTUs were significantly (t-test: p < 0.05) less in the QHL microcosm treatments on Days 7 and 42 than that in their corresponding controls, while a reverse pattern was also observed in the GHL microcosm treatments and controls on Day 42 (Figure S3).

The prokaryotic 16S rRNA gene sequences obtained from the studied samples were mainly affiliated with the Actinobacteria, Alphaproteobacteria, Anaerolineae, Bacteroidia, Betaproteobacteria, Clostridia, Cytophagia, Deinococci, Deltaproteobacteria, Epsilonproteobacteria, Flavobacteria, Gammaproteobacteria, and Ignavibacteria (Figure 3), and the obtained fungal ITS gene sequences were mainly affiliated with the Dothideomycetes, Eurotiomycetes, Microbotryomycetes, and Sordariomycetes (Figure 3). In addition, a large amount (relative abundance >20%) of prokaryotic and fungal sequences were unclassified at the class level in the present study (Figure 3). Relative abundances of the dominant prokaryotic and fungal classes varied with the salinity of the lake microcosms. For example, the relative abundances of Anaerolineae and Betaproteobacteria in the EHL microcosms were higher than those in the QHL and GHL microcosms, while the relative abundances of Gammaproteobacteria in the QHL and GHL microcosms were higher than that in the EHL microcosms, and the relative abundances of Microbotryomycetes in the EHL microcosms were higher than those in the QHL and GHL microcosms, whereas the relative abundances of Dothideomycetes in the GHL microcosms were higher than that in the EHL and QHL microcosms (Figure 3).

Clustering analyses indicated that the prokaryotic and fungal community compositions in the studied microcosms from one lake were clustered together (Figure S4). Within each lake cluster, samples were separated by sampling time (i.e., Days 7 and 42) and types (i.e., initial samples, treatments, and controls) in order (Figure S3), which were also supported by our NMDS analyses (Figure 4) and ANOSIM tests (Table 2). NMDS analyses indicated that samples collected from Days 7 and 42 were separated for each lake, and treatments and controls from each lake were clustered together, respectively (Figure 4). ANOSIM tests showed that the prokaryotic and fungal community structures were significantly (p < 0.05) different between microcosm treatments and their corresponding controls of the studied lakes or between the microcosms sampled on Days 7 and 42 (Table 2).
A total of eight prokaryotic and 11 fungal ZOTUs showed significantly apparent increase in this study (Figure S5), and their taxonomy was shown in Tables S5 and S6. The distributions of these ZOTUs differed among lakes and sampling times. For example, prokaryotic ZOTU30 was only present in the EHL lake microcosms; prokaryotic ZOTU12, ZOTU18, ZOTU33, and ZOTU56 were only present in the QHL microcosms; prokaryotic ZOTU10, ZOTU29, and ZOTU60 were only present in the GHL microcosms. For the fungal ZOTUs, none ZOTU showed apparent increase in the EHL microcosms, while fungal ZOTU16, ZOTU4, ZOTU174, ZOTU87, and ZOTU94 were present only in the QHL microcosms, with the former two appearing in the samples collected on Day 7 and the latter three appearing in the samples collected on Day 42; fungal ZOTU1, ZOTU2, ZOTU5, ZOTU8, ZOTU13, and ZOTU85 were present only in the GHL microcosms, with the former four appearing in the samples collected on Day 7 and the latter two appearing in the samples collected on Day 42 (Figure S5). Since the total abundances of prokaryotic 16S rRNA and ITS genes were higher in the lake treatments than in their corresponding controls, it could be conservatively speculated that the above-mentioned prokaryotic and fungal ZOTUs were stimulated to grow by glucose addition.

**Table 2**

|                  | Prokaryotic community | Fungal community |                  |                  |                  |                  |
|------------------|-----------------------|------------------|------------------|------------------|------------------|------------------|
|                  | EHL       | QHL       | GHL       | EHL       | QHL       | GHL       |
| Group            | R         | P         | R         | P         | R         | P         |
| Day7_ treatment vs. Day7_control | 0.746 | 0.002 | 0.533 | 0.010 | 0.270 | 0.019 |
| Day42_ treatment vs. Day42_control | 0.922 | 0.002 | 1.000 | 0.002 | 0.706 | 0.002 |
| Day7_ treatment vs. Day42_ treatment | 1.000 | 0.002 | 0.994 | 0.003 | 0.819 | 0.002 |
| Day7_ control vs. Day42_control | 0.700 | 0.002 | 1.000 | 0.002 | 0.976 | 0.002 |
3.5. Positive Networks of Prokaryotic and Fungal ZOTUs

Strong positive correlations were observed between the prokaryotic and fungal ZOTUs in the studied microcosm treatments, and they formed different network relationships among the lake microcosms (Figure 5). Specifically, the established networks contained 595, 433, and 543 nodes (Figure S6a), and 2,263, 2,952, and 2,943 edges for the EHL, QHL, and GHL, respectively (Figure S6b). The identified nodes in the EHL, QHL, and GHL networks were dominated by bacterial ZOTUs, and archaeal nodes were more in the QHL network than in the EHL and GHL networks, while fungal nodes were more in the GHL network than in the EHL and QHL networks (Figure S5a). Edges predominately occurred between bacterial taxa in the EHL, QHL, and GHL networks, and the numbers of edges between fungal taxa and archaeal, bacterial or fungal taxa increased with lake salinity (Figure S5b). Furthermore, the average connectivity was 7.61, 13.64, and 10.84 for the EHL, QHL, and GHL networks, respectively. The modularity was 0.57, 0.55, and 0.57 for the EHL, QHL, and GHL networks respectively, and a total of 35, 29, and 32 modules were defined for the EHL, QHL, and GHL networks, respectively.

No network hubs were obtained among the studied lakes in the present study, and most (>97% of total number of nodes) nodes in each network were identified as peripheral nodes (Figure 6). Taxonomically distinct keystone (i.e., module hubs and connectors) prokaryotic and fungal ZOTUs were identified among the networks (Figure 6 and Table S7). For example, there were six module hubs (one archaeal ZOTU and five bacterial ZOTUs) and seven connectors (four bacterial ZOTUs and three fungal ZOTUs) in the EHL network; there were four module hubs (one archaeal ZOTU, two bacterial ZOTUs, and one fungal ZOTU) in the QHL network; there were five module hubs (three bacterial ZOTUs and two fungal ZOTUs) and one bacterial connector in the GHL network (Figure 6 and Table S7).

![Figure 5](image1.png)

**Figure 5.** Correlation-based networks showing the positive associations between the prokaryotic and fungal ZOTUs. The size of node is proportional to the number of edges.

![Figure 6](image2.png)

**Figure 6.** Roles of each nodes being defined by their within-module connectivity (Zi) and among-module connectivity (Pi) in the network.
4. Discussion

Although PEs have previously been documented in lake water columns, few studies reported PEs in lake sediments (Bengtsson et al., 2018). In this study, we reported the occurrences of positive PE induced by glucose in the studied lake sediment microcosms with different salinity and their associated microbial drivers. Our results suggest that positive PEs may generate in natural lake sediments and affect sediment OM mineralization. Such findings are of great importance to understanding of carbon cycling in lacustrine ecosystems.

4.1. Potential Mechanisms Underlying Positive PEs in the Studied Lake Microcosms

Our results suggested that the early (i.e., Day 7) and late (i.e., Day 42) PEs in the studied lake microcosms may be regulated by different microbial mechanisms (i.e., probably stoichiometric decomposition and nutrient mining, respectively). This inference could be supported by the contrasting (i.e., positive and negative) linear relationships between the PE intensities and basal respirations of the studied microcosms on Days 7 and 42 as well as the measured physicochemical parameters (e.g., sediment TN/TP and C:N ratios) of the initial lake sediments in the present study (Razanamalala et al., 2017). Such inconsistency is likely because glucose addition alleviated carbon-nutrient limitation in the studied sediment microcosms during the early incubation, so that microorganisms therein can directly use glucose for growth with the consumption of other available native nutrients (e.g., nitrogen and phosphorus) in the sediments, and they additionally release extracellular enzymes to mineralize the native sediment OM; in contrast, during the late incubation, due to the exhaustion of available nutrients in the studied microcosms, microorganisms may mine native sediment OM through co-metabolisms to obtain nutrients for growth (Blagodatskaya et al., 2014; Chen et al., 2014; Fontaine et al., 2003; Razanamalala et al., 2017). The above speculation still awaits further investigation due to lack of sediment stoichiometry measurements during the incubation in the present study. However, it is still valuable to report such potential mechanisms for PEs in lake sediments.

4.2. Influencing Factors of PE Intensity in the Studied Lake Microcosms

It is remarkable that most of the measured physicochemical parameters exhibited significant influence on the PE intensity in the studied lake microcosms. The influence of salinity on the PE intensity could be ascribed to the following reasons: (1) salinity is one key factor limiting the production of microbial enzymes that take effects in decomposition of OM; (Oren, 2011; Reed & Martiny, 2013) and thus affects the PE intensity through microbial stoichiometric decomposition; (2) salinity affects the interactions (e.g., cooperation) among microbial species (Egamberdieva et al., 2017; Ji et al., 2019; Liu et al., 2017) and thus influences the PE intensity through microbial co-metabolisms; and (3) salinity affects the composition of microbial communities that are involved OM mineralization, thereby influencing the PE intensity. In addition, nutrition-related factors (e.g., TDN, TDP, and C:N ratios) have previously been shown to regulate positive PE in soils (Chen et al., 2018, 2019; Perveen et al., 2019; Razanamalala et al., 2017, 2018). Therefore, it is reasonable to observe the influence of nutrition-related factors on the PE intensity in the studied lake sediments.

4.3. Microbial Drivers of Positive PE in the Studied Lake Microcosms

The positive PEs in the studied lake treatments are likely due to microbial (including prokaryotes and fungi) abundance increase and community composition shifts. The microbial abundance increase may lead to high enzymatic activity for native OM degradation, and microbial community composition shifts may alter and intensify carbon processing functions, both thereby resulting in positive PE (Blagodatskaya & Kuzyakov, 2008; Fang et al., 2018; Mau et al., 2015). Furthermore, the changes of prokaryotic and fungal community compositions owing to glucose addition may suggest that both prokaryotes and fungi may drive positive PEs in the studied lake sediment microcosms. Multiple previous studies have characterized bacterial roles in driving positive PEs (Mau et al., 2015; Morrissey et al., 2017; Razanamalala et al., 2017, 2018). However, little attention has been given to the importance of fungi in positive PEs, especially in lake sediments (Fan et al., 2019; Fontaine et al., 2011). Fungi are widespread and easily form spores stored in lakes (Brandão et al., 2017; Gonçalves et al., 2012; Khomich et al., 2017). Therefore, the input of fresh OM (e.g., glucose) in the studied lake microcosms may stimulate the growth of certain fungal species (e.g., R strategists) that show relative abundance <0.1% in the initial samples but >10 times than before after a short time incubation (Nelson & Carlson, 2012), especially dormant fungi as reported previously, so that their metabolic activity is accelerated, leading to positive PEs through co-metabolism or high enzyme production (Blagodatskaya & Kuzyakov, 2008; Guenet et al., 2010; Kuzyakov, 2010; Mau et al., 2015). Indeed, some
fungal ZOTUs showed apparent increases in abundance as indicated by the qPCR results in the studied lake treatments both on Days 7 and 42 (Figure S5). These above-mentioned observations may suggest that fungi play an important role in driving the early and late PEs in the studied lake sediment microcosms.

In addition, it is notable that microbial contribution to the early and late PEs differed among the studied lake microcosms. Such difference is likely due to different availability of labile substrates (i.e., glucose) between the early and late stages of incubations, thereby selecting for different microbial populations (Razanamalala et al., 2017). Specifically, glucose additions enhance the availability of labile substrates and stimulate the growth of microbial population (e.g., R strategists) that prefer labile substrates at the early stages of incubations; whereas another microbial population (e.g., K strategists who show temperate increase in their relative abundance in response to incubation; Nelson & Carlson, 2012) that are adapted to low availability of labile substrates were dominant at the late stages of incubation when glucose is depleted (Blagodatskaya et al., 2014; Fontaine et al., 2003; Razanamalala et al., 2018). This inference may be possible because we observed a fungal ZOTU4 that showed much higher average relative abundance (50.44% vs. 0.05%) on Day 7 in the QHL treatments than their corresponding control (Figure S4). According to the established definition for R strategists and K strategists by Nelson and Carlson (2012), the fungal ZOTU4 could be considered as R strategist-like taxa, while other prokaryotic and fungal ZOTUs that showed apparent increases in abundance based on the qPCR results of the lake treatments on Day 42 could be considered as K strategist-like taxa. Note that cautions should be taken when defining R and K strategists merely on the basis of microbial abundance (Nelson & Carlson, 2012). Their ecological roles await further investigation.

### 4.4. Potential Microbial Co-metabolisms During the PE Generation

In this study, correlation-based network analyses were employed to discern potential microbial taxa involved in co-metabolisms. Note that correlation analysis does not necessarily mean the real co-metabolisms between two microbial populations during positive PEs (Carr et al., 2019), but it can at least provide some insights concerning potential microbial co-metabolisms, which can be used for further experiment validations.

Our network analyses revealed that archaea, bacteria, and fungi were ubiquitously associated with one another in the studied lake microcosms, suggesting that their co-metabolisms may be partially responsible for the positive PEs in this study. This could be possible because both prokaryotes and fungi can mineralize the recalcitrant OM through ecological cooperation (i.e., co-metabolisms) in the environments, resulting in the positive PE (Blagodatskaya & Kuzyakov, 2008; Chen et al., 2014; Fontaine et al., 2003). It is worthwhile to point out that taxonomically diverse keystone taxa were identified in the studied networks. According to the established ecological perspectives, keystone taxa can be considered as generalists that widely interact with various microbial taxa (Deng et al., 2012; Zhou et al., 2011) and can maintain microbial community structure and function in ecosystems (Banerjee et al., 2018). Therefore, the observed keystone taxa may play important roles in positive PE through co-metabolisms in the studied lake sediment microcosms.

It is also notable that two archaeal keystone taxa (i.e., ZOTU496 and ZOTU647 belonging to *Methanomassiliicoccus* and *Methanobacterium*, respectively) were observed in the EHL and QHL network (Figure 6 and Table S7). *Methanomassiliicoccus* and *Methanobacterium* ever showed syntrophic degradation of OM with other archaea or bacteria (Hirschler-Réa et al., 2012; Kirchman et al., 2014). *Methanomassiliicoccus* species are capable of generating methane using H₂ plus methanol (Dridi et al., 2012), while *Methanobacterium* species can produce methane with the use of H₂ plus CO₂, formate or methanol (Liu & Whitman, 2008). All these substrates are the products of OM mineralization. So the presence of *Methanomassiliicoccus* and *Methanobacterium* suggests that methanogens may be involved in the positive PE in the studied lake sediment microcosms. However, it is still unclear to what extent methanogenic archaea contribute to the positive PE, which awaits further investigation. But any way, our results at least expand the knowledge on microbial drivers of PEs.

Furthermore, it is also remarkable that the numbers of network edges associated with fungal taxa increased with the late PE intensity enhancement among the lake microcosms (Figures 1 and S6b). This finding suggests that fungi and their associated co-metabolisms may play key roles in the late PEs in the present study. This inference was in line with previous studies in soils which suggested that fungal activity is responsible for real PE with co-metabolism of OM in long-term incubations (Blagodatskaya & Kuzyakov, 2008; Fontaine et al., 2011). This may be ascribed to the fact that fungi possess versatile enzymes capable of...
degrading multiple recalcitrant OM, so that they can mine recalcitrant OM to obtain nutrients for other microbial growth when labile OM and environmentally available nutrients were depleted (Fabian et al., 2016; Güsewell & Gessner, 2009).

In addition, our results disclosed that the keystone taxa in the network differed among the lake treatments, and glucose additions stimulated different microbial populations for growth in different lake microcosms (as indicated by Figures 6 and S5 and Table S7). These findings suggest that distinct microbial populations may drive the positive PEs in the different lake microcosms. This can be ascribed to the fact that the original microbial community compositions were essentially different among the studied lakes, and their physicochemical difference (e.g., salinity, pH, TDN and TDP) affects the selection of microbial species during the microcosm incubations (Logares et al., 2013; Oren, 2008; Reed & Martiny, 2013; Wu et al., 2006; Yang et al., 2016).

5. Conclusion

This study confirmed that positive PEs induced by glucose addition can occur in the lake sediments, and both prokaryotic and fungal populations likely contributed to the early and late PEs. In agreement with many previous studies in soils, we found that many physicochemical factors (e.g., salinity, sediment TN, TP, and C:N ratios) significantly influenced the PE intensities in the studied lake sediments. Notably, salinity was shown to significantly affect the early and late PE intensities in the present study: the early PE intensities decreased, while the late PE intensities increased with increasing salinity. Although this study give some novel information in potential microbial drivers and salinity influence on PEs in lake sediments, future work is needed to validate co-metabolisms between prokaryotic and fungal species during PEs in lake sediments.

Conflict of Interest

The authors declared no conflict of interest.

Data Availability Statement

All the raw sequences obtained from this study have been deposited at the NCBI Sequence Read Archive under the project PRJNA377204 (https://www.ncbi.nlm.nih.gov/Traces/study/?acc=PRJNA377204) with accession numbers SRR9696392-SRR9696463, SRR9696702-SRR9696773, and SRR11665933-SRR11665948. Other experimental data were provided in the supplementary materials. All data related to this manuscript has been appropriately provided in FAIR-data compliant repository (https://doi.org/10.5061/dryad.9kd51c5f8).

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