Effects of drought-rewetting process and salinity variations on DOM transformation and bacterial communities in lacustrine sediments

Xiang Zhu
Nanjing Institute of Environmental Sciences

Lei Xie
Nanjing Institute of Environmental Sciences

Yushen Ma
Nanjing Institute of Environmental Sciences

Longmian Wang (✉️ wlmian@sina.com)
Nanjing Institute of Environmental Sciences, Ministry of Ecology and Environment

Qingqing Pang
Nanjing Institute of Environmental Sciences

Fuquan Peng
Nanjing Institute of Environmental Sciences

Zhipeng Xu
Nanjing Institute of Environmental Sciences

Bin Xu
Nanjing Institute of Environmental Sciences

Fei He
Nanjing Institute of Environmental Sciences

Lixiao Ni
Nanjing Institute of Environmental Sciences

Ling Wang
xinjiang tianxi environmental protection technology Co. Ltd

Research Article

Keywords: Drought-rewetting process, Salinity fluctuation, Dissolved organic matter, Fluorescent components, Bacterial communities, Water recharge

Posted Date: January 4th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2374008/v1
Abstract

Climate change and anthropogenic activities cause salinity fluctuations and frequent drought-rewetting process in lacustrine littoral zones. However, joint effects of these processes on quality and quantity of dissolved organic matter (DOM) in pore water and bacterial communities in sediments are not well understood. An experiment was conducted to attempt to fill the above gaps, the results showed that the joint effects of higher salinity (6,000 mg/L) and drought-rewetting process were to retain more humified, more aromatic, and less bioavailable DOM, and to maintain lower DOM concentrations in pore water. The drought-rewetting process magnified influence of salinity on bacterial communities. Before the drought-rewetting process, Hydrogenophilaceae, Oxalobacteraceae, and Flavobacteriaceae participated in the DOM transformation, while Hydrogenophilaceae, Desulfobacteraceae, Anaerolineacea, Planococcaceae, and Clostridiaceae were associated with DOM components after this process. The drought-rewetting process greatly increased significant positive correlations ($P<0.05$) among bacteria, which was consistent with Stress Gradient Hypothesis and destabilized the bacterial communities. Higher (6,000 mg/L) or lower (1,200 mg/L) salinity were all not conducive to stability of bacterial communities in Shahu Lake. The results of this study may provide ideas for further investigating DOM transformation and cycling in brackish-water lakes.

1. Introduction

Dissolved organic matter (DOM) is ubiquitous in natural waters and plays a fundamental role in biogeochemical processes of lakes (Singh et al., 2017), such as cycling of carbon, nitrogen, and phosphorous (Li et al., 2020), fate of organic pollutants and trace metals (Yan and Korshin, 2014), and growth of benthic algae or bacteria (Liu et al., 2021; Gao et al., 2022). Concentrations of DOM in sediment pore water are normally orders of magnitude higher than those in overlying water, serving as an important source of DOM input into water column, especially in shallow lakes (Zhu et al., 2022). Climate change and anthropogenic water recharge have major implications for lake water balance (Jeppesen et al., 2011; Tian et al., 2020), causing fluctuations in water level and salinity and increasing frequency of drought-rewetting in littoral zone, which influences DOM migration and transformation in sediments and affects lake system DOM cycling.

The drought-rewetting process changes redox, physical, and chemical conditions of sediments, thus influencing compositions of DOM (Zhou et al., 2020). Huang et al. (2022b) reported that cycles of drought and wetting favored degradation of labile DOM components and retained components with high aromaticity and molecular size. Solar radiation during drought periods accelerates DOM humification (del Campo et al., 2019), and rewetting initiated anaerobic microbial respiration, transferred electrons to DOM (Lau et al., 2015). In addition, the drought-rewetting process has been proven to influence bacterial communities, a key factor related to quality and quantity of DOM (Gao et al., 2022). Bacterial communities participate constantly in supply and depletion of DOM, which regulates DOM concentration and composition via degradation, absorption, metabolic transformation, and secretion (Xu et al., 2020a; Osterholz et al., 2018). Pozzato et al. (2013) reported that, in the Arabian Sea's oxygen minimum zone,
DOM was processed only by bacteria instead of protozoa and metazoans. Bacterial communities are distinct taxonomically and functionally during drought and wet conditions (Ren et al., 2019) and, consequently, their potential for transforming DOM varies accordingly. Drought conditions caused sediment bacterial community to be dominated by γ and δ-Proteobacteria, which changed capacity for degrading DOM substrates (Freixa et al., 2016), resulting in a longitudinal patchiness of DOM properties and retention rates (Butturini et al., 2016). Rewetting process was reported to increase bacterial activities and enhance content of bioavailable DOM (Ylla et al., 2011).

The salinity of many brackish lakes has become higher in recent decades at a global scale (Xu et al., 2020a), the trend can be alleviated by freshwater recharge (Tian et al., 2020), but the resulting salinity fluctuations play a key role in influencing DOM compositions and bacterial communities in aquatic environments. Higher salinity resulted in DOM with a larger molecular weight, greater degree of oxidation, more lability, lower carbon:nitrogen ratio, lower aromaticity, and increased vulnerability of DOM to degradation by bacteria and light (Xu et al., 2020a; Douglas et al., 2021). As a primary driver of composition and diversity of bacterial communities in water column, salinity has a stronger effect than seasonal changes in many freshwater and saltwater systems (Fortunato and Crump, 2015; Aguirre et al., 2017). Osterholz et al. (2018) found that the diversity of DOM and bacterial communities both decreased along salinity gradient in a temperate estuary. However, a lack of knowledge on the joint effects of salinity increases and drought-rewetting process caused by water recharge on DOM characteristics and bacterial communities in lacustrine sediments is evident. Therefore, influence of water recharge on the above parameters was not considered when measures for brackish-water lakes were formulated in semi-arid areas, may threaten stability of DOM cycling and bacterial diversity.

To fill these gaps, we set up three experimental groups with different salinities and simulated drought-rewetting process, measured the fluorescence and ultraviolet characteristics of DOM in pore water, analyzed variations in sediment bacterial communities using 16 Sr RNA, which is one of the first attempts to ascertain effect of salinity fluctuations and drought-rewetting process on both DOM characteristics and bacterial communities, as well as water recharge measures conducive to stability of DOM components and bacterial communities. The three main objectives are as follows: (1) joint effects of increased salinity and drought-rewetting process on the characteristics of DOM in lacustrine sediment pore water, interactions among bacteria and stability of their communities; (2) response of bacterial communities to DOM components, salinity, and drought-rewetting process; and (3) proper water recharge scheme for brackish-water lakes. The results of this study may provide ideas for further investigating DOM transformation and cycling and developing management measures for brackish-water and saline lakes.

2. Materials And Methods

2.1. Site description
Shahu Lake (106°18′ E, 38°45′ N) is a brackish lacustrine body in Pingluo County, Ningxia Hui Autonomous Region, China (Fig. 1), with a surface area of 13.96 km², a mean and maximum depth of 2.2 m and 6 m, respectively (Chen and Qian, 2017). The climate of this region is characterized by arid or semiarid conditions. The intense evaporation (average 1755.1 mm) and water volumes recharged to Shahu Lake have ranged from 1.79 million to 28.53 million m³ from 2011 to 2017, resulting in fluctuations in salinity and water level (Tian et al., 2020).

2.2. Sample collection and analysis

Sediments from littoral zone and lake water samples were collected in April 2021 at sampling site (106°19′48″ E, 38°48′36″ N) shown in Fig. 1. The water samples were collected by sampling bottles and transferred to the laboratory within 24 h. Physical and chemical parameters of lake water were analyzed in triplicate within 48 h, the results are shown in Table 1. The analytical methods and their corresponding resources, equipment, and detection limits are listed in Table S1. Sediment samples were collected using a shovel and were used for experiment immediately after arriving at laboratory.

2.3. Experimental processes

The experiment was performed in polyvinyl chloride (PVC) cylinders with a diameter of 20 cm and a height of 100 cm. Before the experiment, 5 cm of sediment and 2 L of lake water were added to the cylinders. Salinity of Shahu Lake was 1,200 mg/L during the sampling in 2021 (Table 1), which was 4,434 mg/L in 2014 with conductivity of 6,330 µS/cm (Wu et al., 2017). The salinity in December 2013 may be higher than that in 2014 because the conductivity was 7,593 µS/cm (Tian et al., 2020). Therefore, three experimental groups were set up to simulate salinity fluctuations in Shahu Lake, their salinity values were 1,200 mg/L (S1), 3,600 mg/L (S2), and 6,000 mg/L (S3), and higher salinity were simulated by adding Na₂SO₄, CaCl₂, MgCl₂, and MgSO₄. Three replicates were set up for each experimental group. Period A stimulated the flooding condition of littoral zones for 70 days. The pore water samples were collected at 0, 1, 2, 4, 6, 10, 20, 30, 40, 55 and 70 d using a Rhizon sampler (FLEX, Rhizosphere, Netherlands) and filtered through a 0.45 µm cellulose membrane filter. The sediment samples were collected at 0, 10, 20, 30, 40, 55 and 70 d using a spoon. Lake water with different salinities was slowly replenished down the column wall after sampling, the same amount of sediment was also replenished after sampling. After period A, the sediments were air dried for a month, and then 2 L lake water with different salinities was added to the cylinders to simulated drought-rewetting condition of littoral zones (period B). The period B experiment lasted for 30 days, pore water and sediment samples were collected at 1, 5, 10, 15, 20, 25, and 30 d. The other procedures of period B were similar to those of period A.
### Table 1
The average values of physical and chemical parameters of lake water

<table>
<thead>
<tr>
<th>Number</th>
<th>Parameters</th>
<th>Concentrations</th>
<th>Number</th>
<th>Parameters</th>
<th>Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>8.47</td>
<td>8</td>
<td>NH$_4^+$-N</td>
<td>0.17</td>
</tr>
<tr>
<td>2</td>
<td>DO</td>
<td>6.35</td>
<td>9</td>
<td>Cl$^-$</td>
<td>256.6</td>
</tr>
<tr>
<td>3</td>
<td>Salinity</td>
<td>1200</td>
<td>10</td>
<td>SO$_4^{2-}$</td>
<td>272.4</td>
</tr>
<tr>
<td>4</td>
<td>COD</td>
<td>18</td>
<td>11</td>
<td>Na$^+$</td>
<td>94.0</td>
</tr>
<tr>
<td>5</td>
<td>TP</td>
<td>0.02</td>
<td>12</td>
<td>K$^+$</td>
<td>8.1</td>
</tr>
<tr>
<td>6</td>
<td>TN</td>
<td>0.83</td>
<td>13</td>
<td>Ca$^{2+}$</td>
<td>41.9</td>
</tr>
<tr>
<td>7</td>
<td>NO$_3^-$-N</td>
<td>0.23</td>
<td>14</td>
<td>Mg$^{2+}$</td>
<td>68.1</td>
</tr>
</tbody>
</table>

Units: dimensionless for pH, and mg/L for others.

### 2.4. DOM spectral characteristics and parallel factor analysis (PARAFAC)

The pore water samples were diluted 5 times and analyzed in triplicate. Absorption spectra were recorded between 200 nm and 800 nm at 1 nm intervals by an UV–Vis spectrometer (UV-2550, Shimadzu, Japan). The absorption coefficient is calculated as follows (Keith et al., 2002):

$$\alpha'_{\lambda} = 2.303 \times D(\lambda) / r$$

1

$$\alpha_{\lambda} = \alpha'_{\lambda} - \alpha_{700} \times \lambda / 700$$

2

$\alpha'_{\lambda}$ is uncorrected absorption coefficient at a wavelength of $\lambda$ nm, $D(\lambda)$ is absorbance at a wavelength of $\lambda$ nm, and $r$ is path length (m). $\alpha_{\lambda}$ is corrected absorption coefficient at a wavelength of $\lambda$ nm.

$E2/E3$ and $S_R$ can preliminarily characterize aromaticity and molecular weight of DOM, respectively, which are calculated based on the absorption coefficient as follows (Poulin et al., 2014):

$$E2/E3 = \alpha_{254}/\alpha_{365} \ (3)$$

$$\alpha_{\lambda} = \alpha_{\lambda_0} \times \exp \left[ S \ (\lambda_0 - \lambda) \right]$$

4
\[ S_R = \frac{S_{247-290}}{S_{350-400}} \] (5)

\[ \alpha_{254} \] and \[ \alpha_{365} \] are corrected absorption coefficients at wavelengths of 254 nm and 365 nm, respectively, \( S \) is spectral slope, \( \lambda_0 \) is reference wavelength (generally 400 nm), \( S_R \) is ratio of the spectral slopes, and \( S_{275-295} \) and \( S_{350-400} \) are spectral slopes obtained by fitting the absorption coefficients in wavelength ranges of 275–295 nm and 350–400 nm, respectively.

The three-dimensional fluorescence was measured by a fluorescence spectrophotometer (F-7000, Hitachi, Japan). The excitation wavelengths were scanned from 200 nm to 450 nm at 5 nm intervals, emission scans were collected at wavelengths from 250 nm to 600 nm at 1 nm intervals. PARAFAC was conducted using R 4.0.5 by packages staRdom, dplyr, and tidyr, eliminating primary and secondary Rayleigh scattering regions and correcting the inner-filter effects.

The humification index (HIX) is ratio of average fluorescence intensity from 435 nm to 480 nm, divided by average intensity from 300 nm to 345 nm at a fixed excitation of 254 nm (Mao and Li, 2019). The biological index (BIX) as a DOM source indicator is ratio of emission intensity of 380 nm to 430 nm under excitation of 310 nm (Huguet et al., 2009). The relative concentrations of components identified from PARAFAC are represented by maximum fluorescence intensities (\( F_{\text{max}} \)).

### 2.5. High-throughput sequencing analysis

The DNA of sediment samples was extracted by a DNA Kit (OMEGA, USA). To increase the amount of DNA with few bacteria, homogenization was performed in 15 mL conical vials, then the supernatant was transferred to microcentrifuge tubes for thermal incubation and alkaline lysis. V3-V4 regions of bacterial 16S rRNA were amplified and sequenced using Illumina MiSeq sequencing platform. The detailed analysis methods are shown in appendix S1. The sequencing data were deposited in National Center for Biotechnology Information, the assigned accession number was PRJNA858871.

### 2.6. Data analysis

The one-way analyses of variance (Tukey’s test) and redundancy analysis were carried out using R, network analysis was performed via Gephi and R, and other figures were constructed by Origin 2019.

### 3. Results

#### 3.1. Fluorescence and ultraviolet characteristics of DOM in pore water

As shown in Fig. 2, HIX values of group S1 were lower than those of groups S2 and S3 after the 2nd day in period A and before the 120th day in period B, but differences in HIX among the groups were not significant (\( P > 0.05 \)). HIX values of each group had increasing trends over time in period A and period B; the values on the last day of period A were higher than those on the first day of period B, especially in group S1. The BIX values of group S3 in period A ranged from 0.92 to 1.13, which were significantly lower
than those of group S1 \((P < 0.05)\), between 0.98 and 1.20. The BIX values of group S3 in period B ranged from 0.81 to 1.04, which were significantly lower than those of groups S1 and S2 \((P < 0.05)\), between 0.97 and 1.14. There were no significant differences in \(S_R\) among the groups in either period A or period B \((P > 0.05)\). The \(S_R\) values of each group tended to decrease over time in period A. The \(S_R\) values of groups S1 and S3 in period B were lower than those in period A, and those of group S2 in period B were similar to those on the last day of period A. The E2/E3 values of group S1 were higher than those of groups S2 and S3 in period A, however, differences among them were not significant \((P > 0.05)\). The E2/E3 values in period B were lower than that in period A, and the E2/E3 values in group S3 were significantly lower than those in group S2 \((P < 0.05)\).

### 3.2. Parallel factor analysis

The optimal numbers of DOM components by PARAFAC were based on the results of explained variance, core consistency, split-half, and visual inspection. Four different fluorescent components were identified in pore water of period A (Fig. S1) and period B (Fig. S2). The components of period A were C1, C2, C3, and C4, which of period B were C1b, C2b, C3b, and C4b.

As shown in Fig. 3, C2 and C4 components were dominant in group S1 during the first 10 days and in groups S2 and S3 during the first 4 days in period A. Then, \(F_{\text{max}}\) of C2 and C4 strongly decreased, and C1, C3, and C4 became the dominant components during the rest of period A. The \(F_{\text{max}}\) values of fluorescence DOM components during period B were S1 > S2 > S3. In group S1, \(F_{\text{max}}\) values of DOM components during period B were higher than those on the last day of period A. The dominant components were C1b and C2b on the 100th and 105th days, which were converted to C1b, C3b, and C4b from the 110th day to the 130th day. In groups S2 and S3, \(F_{\text{max}}\) values of the DOM components during period B were similar to those on the last day of period A, and the dominant components were C1b, C3b, and C4b.

### 3.3. 16S rRNA gene sequencing of sediment bacteria

As shown in Fig. S3, dominant bacterial phyla were Proteobacteria and Bacteroidetes in each group during period A. The raise of salinity significantly decreased relative abundances of Bacteroidetes, slightly increased relative abundances of Proteobacteria. After the drought-rewetting process, Proteobacteria, Firmicutes, and Bacteroidetes were dominant in group S1; Proteobacteria, Chloroflexi, and Firmicutes were dominant in group S2; Proteobacteria and Chloroflexi were dominant in group S3. The raise of salinity significantly decreased relative abundances of Bacteroidetes and Firmicutes, significantly increased the relative abundances of Proteobacteria in period B.

The dominant bacterial communities at family and genus levels are shown in Fig. 4 and Fig. S4, respectively. In period A, Hydrogenophilaceae, Oxalobacteraceae, Flavobacteriaceae, Anaerolineaceae, Comamonadaceae, Desulfobacteraceae, Pseudomonadaceae, and Xanthomonadaceae were dominant families in each group. The average relative abundance of Hydrogenophilaceae in S3 was 20.2%, higher
than those in group S2 (16.3%) and group S1 (13.6%). The average relative abundances of Oxalobacteraceae (15.2%), Flavobacteriaceae (12.0%), and Anaerolineaceae (5.0%) in group S1 were higher than those in groups S2 (10.1%, 9.7%, and 4.4%, respectively) and S3 (8.4%, 8.0%, and 4.3%, respectively). *Thiobacillus* was dominant genus of Hydrogenophilaceae, whose average relative abundances in groups S1, S2, and S3 were 12.9%, 15.0%, and 18.9%, respectively. *Massilia* was dominant genus of Oxalobacteraceae, whose average relative abundances in groups S1, S2, and S3 were 14.8%, 9.8%, and 8.2%, respectively. *Flavobacterium* belongs to Flavobacteriaceae, whose average relative abundance in group S1 was 10.4%, higher than that in groups S2 (8.4%) and S3 (6.1%).

The dominant bacteria in period B were different from those in period A, and there were significant differences among groups. In group S1, average relative abundances of Oxalobacteraceae (7.8%), Clostridiaceae (4.6%), Lentimicrobiaceae (4.5%), Bacillaceae (4.2%), and Caulobacteraceae (4.0%) were higher than those of other families, and *Lentimicrobium, Bacillus, and Brevundimonas* were the dominant genera. In group S2, average relative abundances of Anaerolineaceae (12.7%), Hydrogenophilaceae (8.1%), Planococcaceae (6.5%), Desulfobacteraceae (4.1%), and Clostridiaceae (4.0%) were higher than other families. The dominant genera in group S2 were *Thiobacillus* (7.9%) and *Paenisporosarcina* (4.7%), belonging to the families Hydrogenophilaceae and Planococcaceae, respectively. Hydrogenophilaceae (11.3%), Anaerolineaceae (8.6%), Desulfobacteraceae (4.3%), Oxalobacteraceae (3.8%), and Chitinophagaceae (2.8%) were the dominant families in group S3. The dominant genera in group S3 were *Thiobacillus* (11.2%) and *Janthinobacterium* (2.9%), belonging to families Hydrogenophilaceae and Oxalobacteraceae, respectively.

4. Discussion

4.1 Composition of DOM fluorescent components in pore water

C1 was recognized as fulvic acid-like substances with the maximum Ex/Em of 254/425 nm (Santín et al., 2009). C2 displayed a single Ex/Em peak of 270/315 nm and was assigned to protein-like structures similar to tyrosine (Murphy et al., 2011). C3 exhibited two peaks at 235 nm/354 nm (Ex/Em) and 275 nm/354 nm (Ex/Em), which were classified as tryptophan-like substances (Yang et al., 2015). C4 components were identified at Ex/Em wavelengths of 225/300–400 nm, which resembled aromatic proteins (Huang et al., 2022a).

C1b (Ex/Em = 235/412 nm) was similar to C1 and was categorized as a fulvic acid-like substance. C2b (Ex/Em = 225,275/320 nm) were tyrosine-like substances (Cheng et al., 2018). C3b comprised of two peaks at Ex/Em wavelength pairs of 264/445 nm and 355/445 nm, implying humic-like compounds with relatively high molecular weights (Wheeler et al., 2017). C4b had a primary fluorescence peak at an Ex/Em wavelength pair of 247/390 nm and a secondary peak at 298/390 nm, indicating microbial humic-like substances, which were relatively aliphatic and with low molecular weight (Murphy et al., 2011).
4.2 Influence of drought-rewetting process and salinity variation on DOM fluorescent components

Rewetting after drought resulted in lower HIX in pore water (Fig. 2), indicating that this process decreased humification of DOM, which was associated with increasing proportions of tyrosine-like components on the first day of period B (Fig. 3). Firstly, the drought period inhibited bacterial activities and utilization of protein-like substances and increased production of extracellular polymeric substances, including protein-like DOM (Gionchetta et al., 2019). Secondly, the drought-rewetting process resulted in higher preferential adsorption of carboxyl over amide, and fewer tyrosine-like components were adsorbed on the clay surfaces (Olshansky et al., 2018). Rewetting was reported to increase bioavailability of DOM and bacterial activities because occluded carbon sources were liberated for fast microbial reactivation (Ylla et al., 2011; Gionchetta et al., 2019), thus, higher BIX values were observed on the first day of period B than on the last day of period A (Fig. 2). The decreasing trend of BIX in period B may because that labile DOM was gradually exhausted. Notably, values of $S_R$ and $E_2/E_3$ decreased after the drought-rewetting process (Fig. 2), indicating an increase in the aromaticity and molecular weight of DOM, which was linked with the significantly increased $F_{\text{max}}$ of humic-like components (Fig. 3). Huang et al. (2022b) found that cycles of drought and wetting were conducive to DOM components becoming more aromatic, hydrophobic, and humified because the labile fractions were utilized and/or transformed to aromatic structures by the bacterial communities. Yamashita and Tanoue (2003) also considered that newly produced humic-like components may represent degradation products of amino acids and protein-like components by microbial metabolism. Large sized polysaccharides secreted by bacteria during drought period that may also account for increase in DOM molecular weight (von Schiller et al., 2015). In addition, direct solar radiation on sediments and relevant heat during drought conditions accelerated the humification of DOM by oxidative polymerization reactions (del Campo et al., 2019). The $F_{\text{max}}$ values of DOM components were observed to greatly increased after the drought-rewetting process in group S1. It was reported that moisture of sediment led to formation of cracks during drought conditions, which increased surface area and oxygen availability of sediments and promoted aerobic destruction of organic matter, thus leading to more DOM release (Schiebel et al., 2019). However, there was little influence of the drought-rewetting process on $F_{\text{max}}$ values of DOM components in groups S2 and S3, suggesting that higher salinity inhibited the release processes or mobilized the DOM from pore water to surface water.

The HIX values of S1 were lower than those of S2 and S3 during period A, and $S_R$ and $E_2/E_3$ had the opposite regularities, indicating that higher salinity of lake water caused DOM in pore water to become more aromatic and humified with a higher molecular weight. Previous studies reported that an increase in salinity caused DOM to show lower aromaticity in surface water (Xu et al., 2020a; Douglas et al., 2021), which was different from the results of this experiment, probably because the higher salinity may have facilitated photodegradation of aromatic components in surface water, while these components in pore water were not influenced by light. Significantly lower BIX values were observed in S3 ($P<0.05$), indicating that increase in salinity decreased autochthonous DOM and inhibited effect of bacteria on DOM composition. The protein-like fractions were considered to be preferentially consumed during
biodegradation (Zhou et al., 2019), as shown in Fig. 3. These processes were accelerated at high salinity, especially biodegradation of tyrosine-like components and aromatic proteins.

The joint effects of salinity increase and drought-rewetting process were to make the more humified, more aromatic, less bioavailable DOM remain in pore water, and to preserve DOM concentrations at lower levels, especially when the salinity was at the concentration of 6,000 mg/L, which influenced cycling and utilization of carbon in brackish-water lake, and further affected the lacustrine ecosystem.

4.3 Response of bacterial relative abundances in sediments to DOM components, salinity, and drought-rewetting process

It has been reported that drought-rewetting process caused rupture of many cells by osmotic shock, especially bacterial communities dominated by Proteobacteria (Schimel et al., 2007; Marxsen et al., 2010). In this study, decrease of Shannon indexes and relative abundances of Proteobacteria after drought-rewetting process were observed (Fig. S5), the values had not been recovered to levels before this process by the end of the experiment. Different from the previous work, relative abundances of Proteobacteria in group S3 were similar before and after the drought-rewetting process, likely due to its resistance to salinity (Chen et al., 2017) and stress of salinity on many other phyla. Moreover, the drought-rewetting process enhanced the differences of in bacterial communities among groups. As shown in Fig. S5, there were no significant differences of Shannon indexes among groups before drought-rewetting process, but Shannon indexes in S1 were significantly lower than those in S2 (P < 0.05) after this process, indicating that this process magnified the influence of salinity on bacterial communities, may result in greater differences of DOM at different salinity.

According to Fig. 5a, Hydrogenophilaceae showed positive correlations with salinity and F_{max} of C1 and C3 and negative correlations with F_{max} of C2 and C4 in period A. *Thiobacillus* was the dominant genus of Hydrogenophilaceae, which could adapt to high salinity environment (Xu et al., 2020b). *Thiobacillus* could degrade organic matter containing sulfur (Wang et al., 2018), probably producing fulvic acid-like substances and contributing to higher HIX and lower S_R and E2/E3 under higher salinity. *Thiobacillus* decreased pH by sulfur oxidation, inducing release of DOM indirectly (Zhu et al., 2022) and secreted extracellular polymeric substances containing proteins (Ye et al., 2021), accounting for the relationships between relative abundances of Hydrogenophilaceae and F_{max} of protein-like components. The relative abundances of Oxalobacteraceae were negatively correlated with salinity, indicating that this family was not tolerant to high salinity. Moreover, the relative abundances of Oxalobacteraceae were positively correlated with F_{max} of C2 and C4 and negatively correlated with F_{max} of C1 and C3. *Massilia* was the dominant genus of Oxalobacteraceae and could biodegrade refractory DOM to compounds with low complexity and aromaticity (Qiao et al., 2021), suggesting that this genus degraded fulvic acid-like components. *Massilia* was reported to produce indole derivatives by utilizing tryptophan (Agematu et al., 2011), which was one of reasons for negative correlation between relative abundances of...
Oxalobacteraceae and $F_{\text{max}}$ of C3. The correlations between Flavobacteriaceae and salinity and $F_{\text{max}}$ of four DOM components were similar to those of Oxalobacteraceae because the dominant genus of Flavobacteriaceae was *Flavobacterium*, which could utilize amounts of dissolved proteins and degrade macromolecular organic matter (Ye et al., 2020). Anaerolineaceae, Comamonadaceae, Desulfobacteraceae, Pseudomonadaceae, and Xanthomonadaceae had relatively little influence on DOM components during period A.

After drought-rewetting process, interaction patterns between dominant bacteria and DOM components were greatly changed. As illustrated in Fig. 5b, Hydrogenophilaceae showed a negative correlation with four DOM components, which was different from that before drought-rewetting process, because of drought stress and alteration of sediment environments. Correlations between sulfate-reducing bacterium Desulfobacteraceae and salinity and $F_{\text{max}}$ of DOM components were similar to those of Hydrogenophilaceae, likely because of its positive correlation with *Thiobacillus*. Anaerolineaceae with strong resistance to hypersaline conditions due to protection of extracellular polymeric substances (He et al., 2020) and acted as a primary degrader for refractory DOM, such as humic-like substances (Zhang et al., 2018). Thus, their relative abundances were positively correlated with salinity and negatively correlated with $F_{\text{max}}$ of C1b, C3b, and C4b. Planococcaceae was positively correlated with $F_{\text{max}}$ of C1b and C2b, suggesting that Planococcaceae participated in the production of fulvic acid-like and tyrosine-like substances. Clostridiaceae is a proteolytic bacterium that utilizes protein as its preferred substrate (Kim et al., 2020) and produces fulvic-like, humic-like, and tyrosine-like components during the biodegradation of proteins, resulting in positive correlations with $F_{\text{max}}$ of these components. Oxalobacteraceae, Caulobacteraceae, and Chitinophagaceae had relatively little influence on DOM components during period B.

Salinity variations and the drought-rewetting process greatly altered structures of bacterial community, probably due to stress and lack of labile carbon sources. Bacterial community structure was correlated with its functions (Riah-Anglet et al., 2015), thus the alteration of bacterial communities, especially dominant bacteria associated with DOM components, was one of crucial reasons for variation of DOM compositions in pore water.

### 4.4 Correlation among the dominant families under drought-rewetting process and salinity gradient

As shown in Fig. 6, there were 24 significant positive correlations and 25 significant negative correlations ($P < 0.05$) among dominant families in period A, there were 38 significant positive correlations and 25 significant negative correlations ($P < 0.05$) among dominant families in period B. The drought-rewetting process increased the positive correlations among the bacteria. The results of this study support Stress Gradient Hypothesis: competitive interactions decrease and facilitative interactions increase along the stress gradient (Bertness and Callaway, 1994). Previous studies have demonstrated that environmental stress increased facilitation among bacteria (Piccardi et al., 2019; Hernandez et al., 2021). In this study,
bacteria may conduct metabolic cross-feeding and interactions among them switch from competition to facilitation when labile DOM is depleted (Goldford et al., 2018), which can be supported by increase of positive correlations between Hydrogenophilaceae and other bacteria after drought-rewetting process. It was worth noting that Hydrogenophilaceae was dominant family and was correlated with DOM components. Romdhane et al. (2021) found that modified bacterial interactions greatly changed cycling of nitrogen and carbon in soil. The alternation of the interactions between Hydrogenophilaceae and other bacteria may affect its role on DOM transformation and cycling. Moreover, decrease in ratio of negative and positive correlations among bacteria results in bacterial communities becoming more precarious, because the bacteria support each other and perturbations on any of them can easily influence the whole system (Coyte et al., 2015). Therefore, drought-rewetting process destabilized the bacterial communities in sediments, which may further threaten the stability of DOM cycling.

As shown in Fig. S6, significant positive correlations ($P<0.05$) among dominant families were 27 in group S1, which were 31 and 35 in groups S2 and S3, respectively. Significant negative correlations ($P<0.05$) were 19, 35 and 25 in groups S1, S2 and S3, respectively. Increase of facilitative interactions were observed along the salinity gradient, however, numbers of competitive interactions did not show a linear change under increase of salinity. It was interesting that highest negative:positive correlation was observed in group S2, the value of negative:positive correlation in group S1 was similar to that in group S3, indicating that bacterial communities was more stable under salinity of 3,600 mg/L, higher or lower salinity probably both destabilized bacterial communities in sediments of Shahu Lake. Fluctuations of salinity also affected stability of bacterial communities linked with DOM transformation. For example, significant positive correlations ($P<0.05$) between Hydrogenophilaceae and other bacteria were 6, 6, and 4 in groups S1, S2 and S3, respectively. Significant negative correlations ($P<0.05$) were 4, 2, and 0 in groups S1, S2 and S3, respectively. Hydrogenophilaceae was resistant to salinity and raise of salinity increase its relative abundances in this study, however, salinity increase diminished its interrelationships with other bacteria and destabilize its communities, may indirectly threaten bacterial diversity and DOM cycling.

### 4.5 Water recharge scheme and management measures for brackish-water lakes

Exorbitant salinity and drought-rewetting process resulted in less protein-like substances and more humic-like components in pore water, thus reducing pool of bioavailable DOM, destabilizing bacterial communities in brackish-water lake, which was harmful to the utilization of carbon or nutrients and may deteriorate water quality. It was worth noting that excessive low salinity is also not conducive to the stability of bacterial communities in brackish-water lakes. Water recharge is essential measure to maintain water balance of inland lakes, take Shahu Lake as an example, annal volumes of water recharge should exceed 22.09 million m$^3$ based on average evaporation and precipitation, but exorbitant water recharge also destabilizes the bacterial system. To maintain the stability of salinity and water level, continuous recharge of water with lower salinity to brackish-water lakes were appropriate instead of
intermittent recharge. Important problems are that it is difficult to determine volumes of water recharge, and water recharge only alleviate raise of salinity concentrations in the short term, but does increase salinity loading. Thus, it is necessary to monitor salinity, dynamically change water recharge volumes based on salinity, and strengthen water exchange between brackish-water lakes and other water bodies. In addition, buffer strips construction, saline-alkali soil remediation, and prevention of agricultural return flow draining into lake or water recharge canals were also effective measures to avoid excessive increase of salinity and frequent drought-rewetting process in brackish-water lakes.

5. Conclusions

The rewetting after drought and higher lake water salinity caused DOM in pore water to become more aromatic, humified, and less autochthonous. Before the drought-rewetting process, Hydrogenophilaceae, Flavobacteriaceae and Oxalobacteraceae were dominant bacteria associated with DOM transformation, which varied to Hydrogenophilaceae, Desulfobacteraceae, Anaerolineacea, Planococcaceae, and Clostridiaceae after the process. Drought-rewetting process resulted in more significant positive correlations ($P < 0.05$) among bacteria, especially correlations between Hydrogenophilaceae and other bacteria. Lack of bioavailable DOM may be an important reason for increase of bacterial facilitation, which destabilized the bacterial communities. Higher ($6,000$ mg/L) or lower ($1,000$ mg/L) salinity are not conducive to stability of bacterial communities in Shahu Lake. Monitoring salinity and continuous water recharge with dynamically changing volumes may be effective measures for weakening impact of drought-rewetting process and salinity fluctuations on DOM transformation and microecosystem balance in brackish-water lakes.

Declarations

Fundings

This work was supported by the Innovative team project of Nanjing Institute of Environmental Sciences, MEE (GYZX200101), Key R & D Program of Ningxia Hui Autonomous Region (2021BEG01002, 2019BFG02028), the National Natural Science Foundation of China (52270160), Xinjiang Uygur Autonomous Region Science and Technology Program Plan (2022E02026).

Author Contributions

Xiang Zhu and Lei Xie: Conceptualization, Methodology, Formal analysis, Data curation, Software, Writing-Original draft, Visualization. Yushen Ma, Qingqing Pang, Fuquan Peng, and Zhipeng Xu: Data curation, Software, Investigation, Visualization. Bin Xu, Lixiao Ni and Ling Wang: Investigation, Resources, Data curation, Supervision. Longmian Wang and Fei He: Conceptualization, Methodology, Validation, Resources, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Data availability: The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.
Ethics approval: Not applicable.

Consent to participate: Not applicable.

Consent for publication: Not applicable.

Competing interests: The authors declare no competing interests.

References


49. Xu XC, Zhang R, Jiang HB, Yang FL (2020b) Sulphur-based autotrophic denitrification of wastewater obtained following graphite production: Long-term performance, microbial communities involved,


Figures
Figure 1

Location of Shahu Lake and sampling site
Figure 2

Fluorescence and ultraviolet characteristics of DOM under different salinities in periods A and B: (a) HIX, (b) BIX, (c) $S_R$, (d) $E_2/E_3$; different letters in brackets indicate significant differences at $P<0.05$ according to Tukey’s test.
Figure 3

$F_{\text{max}}$ of different DOM components along salinity gradient in periods A and B: (a) S1, (b) S2, and (c) S3
Figure 4

Bacterial community compositions at family level: (a) S1, (b) S2, (c) S3
Figure 5

Redundancy analysis between relative abundances of dominant families and DOM components and salinity in period A (a) and period B (b)
Figure 6

Network constructed around main bacterial families based on Spearman rank correlation coefficients during period A (a) and period (b). Green indicates a significant positive correlation ($P<0.05$), and red indicates a significant negative correlation ($P<0.05$); size of each node is proportional to number of connections.
Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supportingdata.docx