Diversity of eukaryotic micro-organisms and changes in the dominant fungal taxa composition in relationship with soil environment in the Ebinur Lake wetland

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ABSTRACT
Four different soil layers in three sampling sites in the Bortala and Jinghe River basins of the Ebinur Lake Wetland Nature Reserve were collected at different time points during April, July and October in 2012. The sampling sites were: the main inlet lake of the Jinghe River in the Lake District of the Bird Island Protection Station (SP1), the meadows of the Bird Appreciation Station near the main inlet lake in Bole (SP2) and the salt works in the overflow area of the main inlet lake in Jinghe (SP3). The diversity and phylogenetic analyses of the 18S rDNA sequences of eukaryotic micro-organisms were performed using DGGE. The diversity of eukaryotic micro-organisms and the correlation between community composition and soil environment factors were analysed using the SPSS software. The diversity index calculated using the DGGE analysis showed the following pattern: SP1 > SP3 > SP2. The community composition of eukaryotic micro-organisms in the Ebinur Lake wetland was different in the Jinghe and Bortala River basins. In this wetland, the diversity of eukaryotic micro-organisms was mainly affected by the extent of soil fertility: the higher the soil fertility, the lower the eukaryotic microbial diversity. In contrast, the barren soils were associated with higher microbial diversity. Since the soil nutrients present in the Ebinur Lake wetland were low, the wetland fertility was also low. The community composition of eukaryotic micro-organisms in the Ebinur Lake wetland was mainly affected by the soil water content, pH, salinity, nutrient content and presence of heavy metal ions.

INTRODUCTION

The Ebinur Lake wetland located in Xinjiang is typically representative of the inland lakes in the arid zones of China. It is a combination of lacustrine, palustrine and riverine wetlands, which are the characteristic wetland types [1]. The Bortala and Jinghe Rivers are important sources of water for the Ebinur Lake. The changes in these rivers result in dynamic changes in the Ebinur Lake and also affect the Ebinur Lake basin in the northern part of Xinjiang. Due to the living environment of people, coupled with the impact of perennial winds from the Ala mountain pass, dusty weather has become increasingly common [2].

Eukaryotic micro-organisms are a part of important biochemical processes such as ammonification, nitrification and cellulose decomposition. Studying the eukaryotic microbial diversity will help us in understanding these processes and applying them effectively to our lives for the benefit of mankind. Understanding the variations in characteristics of diverse soil eukaryotic micro-organisms over temporal and spatial scales, the correlation between the dominant microbial population composition and soil environment factors, and spatial distribution and composition of the dominant flora in a particular environment provides the theoretical basis for environmental monitoring, early warning and evaluation [3].

At present, the major problems that the Ebinur Lake Wetland Nature Reserve faces are the decline in soil fertility and severe desertification. Meanwhile, due to the rapid industrialization and population growth, the land under construction is increasing. In addition, land development planning lacks unified river basin management [4]. In recent years, due to predatory development practices and soil utilisation, the Ebinur Lake wetland is gradually moving in the direction of desertification and salinisation. The study of dynamic changes in the soil microbial community reflects the combined effects of environmental impact on edaphic factors. These factors strongly influence the ecological distribution of soil microbes. Edaphic factors (such as soil type, salinity, nutrient content and extent of agricultural use), along with climatic conditions, have a considerable effect on the ecological distribution of microbes in saline soil. Studying the correlation between soil eukaryotic micro-
organisms and the physical and chemical properties of soil is of immense significance [5].

In this paper, the 18S rDNA sequences of eukaryotic micro-organisms isolated from four different soil layers present in three sampling sites of Bortala and Jinghe River basins in the Ebinur Lake Wetland Nature Reserve, were isolated and analysed using denaturing gradient gel electrophoresis (DGGE). The microbial diversity and phylogenetic analyses were performed based on the DGGE band patterns [6,7]. The diversity of eukaryotic micro-organisms, and the correlation between community composition and soil environment factors were analysed using the SPSS software. The dynamics of eukaryotic microbial communities and the composition of dominant microflora in the Ebinur Lake wetlands were analysed [8,9].

Materials and methods

Sample collection

The Ebinur Lake wetland is located in the North-Eastern part of Bole city in Xinjiang Uyghur Autonomous Region (longitude, 82° 36′–83° 50′ East; latitude, 44° 37′–45° 15′ North). It is the national lake of the western part of China. It also forms the lowest depression and acts as a water gathering centre for the South-Western part of the Junggar basin. The Ebinur Lake wetland has a typical arid temperate continental climate which is dry and windy. It is characterized by adequate sunlight, large temperature differences between summers and winters, and considerably less rainfall. In this study, a multi-point sampling method was adopted. Three sampling sites located at 300-m intervals in the Ebinur Lake Wetland National Nature Reserve were selected from the main inlet lake of the Jinghe River in the Lake District of the Bird Island Protection Station near the main inlet lake in Bole (SP1), the meadows of the Bird Appreciation Station in the main inlet lake in Bole (SP2) and the salt works in the overflow area of the main inlet lake in Jinghe (SP3)(Table 1). The samples were taken during the months of April, July and October in 2012. At each sampling site, three pits were randomly chosen. The vegetation from the surface layer was shaved, and the soil samples were collected from depths of 0–5, 5.1–15, 15.1–25 and 25.1–35 cm. The samples procured from the same depth of a particular sampling site were evenly mixed, transferred to an aseptic bag, and labelled appropriately. The bags were stored in a portable refrigerator. Subsequently, microbial DNA was extracted.

Analysis of the physical and chemical properties of the soil samples

The soil water content was determined by the oven drying method at 105°C. pH was measured by the potential method, using the PHS-3C pH meter (Model PHS-3C pH Meter, Shanghai INESA Scientific Instrument Co. Ltd., Shanghai, China). Salinity was measured by the conductivity method, using the DJ-320 conductivity meter. Determination of organic matter with potassium cyclo-pate method—external heating method, alkaline hydrolysis nitrogen content determination by alkali solution diffusion method. The available phosphorus content in soil was determined by subjecting the sodium bicarbonate-extracted soil samples to the molybdenum—antimony colorimetric method. The available potassium content was determined for the ammonium acetate extracts of soil using the flame photometric method.

DNA extraction and amplification of the 18S rDNA sequences using PCR

The soil samples were removed from the refrigerator and gradually thawed from −20 to 4°C. In order to improve the DNA extraction efficiency, the samples were washed with a pre-lysis buffer, before the extraction procedure [10]. The 18S rDNA sequences were amplified using the primers Euk1A (5’-TCCGTAGGTGGCATCTGCGG-3’), Euk516r (5’-ACCAGACTTGCCCTCC-3’) and GC hairpin (5’-CGGCCGGGCGCGCCGCCGGCGGGGCACGGGGGG-3’) [9]. The amplified fragment corresponded to 560 bp. The PCR reaction (total volume, 50 μL) was setup using the 2 × PCR Mix (20 μL), DNA template (6 μL), forward primer (10 μmol/mL, 1.5 μL) and ddH2O (21 μL). The amplification conditions were as follows: initial denaturation at 94°C for 130 s, followed by 35 cycles at 94°C for 30 s, 57°C for 45 s and 72°C for 130 s, and final extension at 72°C for 10 min. After PCR, the samples were maintained at 4°C.

Table 1. Description of the sampling sites in the Ebinur Lake Wetland.

| Sample plot | Site | Longitude/Latitude | Height/m | Main plant type |
|-------------|------|--------------------|----------|-----------------|
| SP1         | Bird Island Protection Station in Jinhe’s Main Lake Inlet | 82°48’51.6"E–82°49’30.0"E, 44°50’13.5”N–44°50’11.5”N | 192 | Tamax shrub, salt festival wood |
| SP2         | Meadow area of Bird Appreciation Station in Bole’s Main Lake Inlet | 82°41’33.2’E–82°41’11.1’E, 44°51’42.3”N–44°51’49.6”N | 194 | Reed meadow |
| SP3         | Salt-works of overflow area in Jinhe’s Main Lake Inlet | 82°35’50.2’E–82°56’15.8”E, 44°47’26.2”N–44°47’17.0”N | 189 | Desert salt grass |
**DGGE analysis and DNA sequencing**

DGGE was performed using the DCode™ Universal Mutation Detection System (Bio-Rad, CA, USA). The PCR products were electrophoresed using a polyacrylamide gel (6%) containing a linear gradient (20%–45%) of the denaturant, in Tris-acetate-EDTA (TAE) buffer (1 x). The concentration of denaturant increased from top to bottom in the gel. The samples were electrophoresed at constant voltage (80 V) and temperature (60 °C) for 12.5 h, and subjected to silver staining [11,12]. Images of the stained gels were captured using the Powerlook 2011XL colour scanner. The extracted DNA was amplified by PCR using the primers Euk1Af and Euk516r without the GC hairpin structure according to the 18S rDNA amplification protocol [9,13]. The amplification products were detected as single bands on agarose gel (1%). The DNA bands were sequenced by Beijing three Bo Polygalaceae Biotechnology Co. Ltd.

**Sequence comparison and phylogenetic tree construction**

The 18S rDNA sequences were obtained from GenBank, similarity searches were performed using BLAST and the sequence with the highest similarity was downloaded. The DNA sequences were aligned using Clustal X [14]. The neighbour-joining (N-J) method was used to construct the phylogenetic tree by MEGA software [15,16]. The nucleotide sequences obtained in this study were deposited in the GeneBank database under accession numbers KY295854–KY295888 and KY295890 for 18SrDNA.

**Statistical analysis**

The DGGE profiles of DNA isolated from soil samples collected from different sampling sites at different times were processed using the Quantity One analysis software (Bio-Rad). The UPGMA method was used for clustering similar bands in the DGGE profiles. The Shannon–Wiener (H), evenness (E), richness (S) and Simpson (D) indices were used to evaluate the diversity of eukaryotic micro-organisms present in the Ebinur Lake wetland of Xinjiang.

**Results and discussion**

**Community structure of eukaryotic micro-organisms in different soil layers and seasons in Ebinur lake wetland**

This study revealed the presence of the eukaryotic micro-organisms in a desert wetland ecosystem in different seasons. Figure 1 shows the distribution pattern of eukaryotic micro-organisms in the soil samples collected from sampling sites SP1, SP2 and SP3 at depths of 0–5, 5–15, 15–25 and 25–35 cm, in the months of April, July and October. The DGGE patterns of 18S rDNA sequences of soil micro-organisms are shown in Figure 1. DGGE revealed that soil samples could be differentiated based on the number of bands, their location, intensity and mobility. The diversity of eukaryotic micro-organisms present in each sampling site was demonstrated. Through the analysis, we found that the number of bands on the gel and the strength of the existence of a certain difference in the same depth of soil and different sampling time. During the same sampling time, the number and the brightness of bands of samples from different soil depths are different. This indicated that the dynamic succession of eukaryotic microbial communities and the composition of dominant microbes were related to the sampling time and soil depth.

The similarity analysis of the distribution of the bands in each lane (shown in Table 2(A)) shows that the similarity between all samples is between 40.3% and 72.7%, indicating that the eukaryotic microbial community structure between the samples have similarities and differences. The similarity between sample No. 5 and sample No. 8 was high (72.7%), whereas between No. 1 and No. 11, the similarity was the lowest – only 40.3% (Table 1(A)). The community structure of the eukaryotic micro-organisms in SP1 was similar between two adjacent middle soil layers at the same sampling time and the difference was small, while in 0–5 cm superficial soil layer and 25–35 cm deep soil, the similarity was lower and the difference was large. The similarity of all the samples was between 25.9% and 65.0%, indicating that there were some differences in the structure of the eukaryotic microbial community between the samples (Table 2(B)). No. 4 and No. 5 had the highest similarity of 65%. The similarity of No. 3 and No. 4 was the lowest, only 25.9%. The results showed that the variation of eukaryotic microbial community structure in different sampling times was lower than that in different soil layers. As it can be seen from Table 2(C), the similarity between all the samples was 31.4%–74.6%, which indicated that there were some differences in the community structure of soil eukaryotic micro-organisms in SP3 samples. The similarity of No. 3 and No. 6 was the highest – 74.6%. The similarity of No. 3 and No. 7 was the lowest, only 31.4%. The results showed that the eukaryotic microbial community structure of the SP3 plots in the Ebinur Lake wetland had a large difference between the upper soil and the deep soil layers of 0–15 cm, and the eukaryotic microbial community structure of different soil layers was different at different sampling times.
is, the community structure of soil eukaryotic microorganisms was affected by the sampling time and soil depth.

The results showed that the diversity index calculated from the DGGE analysis had the following pattern: SP1 > SP3 > SP2. This indicated that the diversity of soil eukaryotic micro-organisms in the Jinghe River basin was higher, compared to that in the Bortala River basin. The analysis also revealed the dynamic succession of the eukaryotic microbial community in the Ebinur Lake wetland and the changes in the composition of dominant micro-organisms.

Figure 1. Denaturing gradient gel electrophoresis (DGGE) analysis for evaluating the diversity of soil eukaryotic micro-organisms in the SP1, SP2 and SP3 sampling sites.
Cluster analysis

Figure 2 represents the similarities between the DGGE bands. In order to understand the distribution of eukaryotic micro-organisms at three different sampling times and four different soil depths, the bands in the DGGE profiles were analysed using the UPGMA algorithm. The eukaryotic microbial community at the SP1 sampling site was mainly clustered into two groups. The results showed that the community structure of eukaryotic micro-organisms was similar in 0–35 cm soil layer. However, in April [at the 5–15 and 15–25 cm soil depths (No.4 and No.7)] and in July [(No.5 and No.8) at the 5–15 and 15–25 cm soil depths], considerably less difference in the community structure of eukaryotic micro-organisms was observed between adjacent soil layers at the same sampling time. The eukaryotic microbial community structure of the SP2 sampling site clustered into three main groups. The same soil sample at different sampling times revealed highly similar community structure of eukaryotic micro-organisms. Additionally, different soil samples at the same sampling time also showed a correlation between the community structures of eukaryotic micro-organisms. The 18S rDNA bands indicating the eukaryotic microbial diversity in the SP3 sampling site were mainly clustered into four categories on the DGGE map. Among them, SP3 of 15–25 cm in April (No.7) were divided into one class and the samples of 25–35 cm soil layers in July and October (No.11 and No.12) were clustered together; grades 8 and 10 were preferentially polymerized, then combined with 5. The community structure of the soil eukaryotic microbes showed high similarity among the same soil samples at different sampling times.

Analysis of eukaryotic microbial diversity in soils

In order to quantitatively analyse the diversity of eukaryotic micro-organisms present in the Ebinur Lake wetland, the Shannon–Weiner, evenness, richness and Simpson indices of each soil sample were calculated, based on the number of DNA bands per lane and their relative brightness. The results shown in Table 3 revealed that

| Lane | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|------|---|---|---|---|---|---|---|---|---|----|----|----|
| A    |   |   |   |   |   |   |   |   |   |    |    |    |
| SP1  | 1 | 100|   |   |   |   |   |   |   |    |    |    |
|      | 2 | 49.1|   |   |   |   |   |   |   |    |    |    |
|      | 3 | 44.7|   |   |   |   |   |   |   |    |    |    |
|      | 4 | 49.3| 60.2| 64.8| 100|   |   |   |   |    |    |    |
|      | 5 | 52.1| 56.0| 61.5| 63.3| 100|   |   |   |    |    |    |
|      | 6 | 48.6| 59.3| 72.4| 67.1| 64.8| 100|   |   |    |    |    |
|      | 7 | 47.1| 48.7| 41.3| 52.3| 53.4| 50.2| 100|   |    |    |    |
|      | 8 | 53.1| 65.6| 65.6| 66.6| 72.7| 67.1| 50| 100|    |    |    |
|      | 9 | 53.3| 60.8| 57.7| 55.0| 59.1| 61.5| 49.6| 66.5| 100|    |    |
|      | 10| 41.8| 64.8| 58.0| 51.6| 60.2| 65.6| 44.4| 64.6| 57.9| 100|    |
|      | 11| 40.3| 69.6| 66.5| 59.1| 60.9| 66.5| 46.4| 69.3| 63.3| 71.5| 100|
|      | 12| 40.7| 49.9| 53.4| 54.8| 57.4| 68  | 47.9| 57.9| 56.2| 60.2| 56.7| 100|
| B    |   |   |   |   |   |   |   |   |   |    |    |    |
| SP2  | 1 | 100|   |   |   |   |   |   |   |    |    |    |
|      | 2 | 59.5|   |   |   |   |   |   |   |    |    |    |
|      | 3 | 42.7|   | 49 | 100|   |   |   |   |    |    |    |
|      | 4 | 54.8| 48.5| 25.9| 100|   |   |   |   |    |    |    |
|      | 5 | 55.4| 58.9| 33.9| 65 | 100|   |   |   |    |    |    |
|      | 6 | 55.6| 53.7| 35.9| 48.2| 59.6| 100|   |   |    |    |    |
|      | 7 | 54.3| 48.7| 32.8| 63.4| 64.8| 57.7| 100|   |    |    |    |
|      | 8 | 57.9| 44.6| 32.1| 57.2| 60.2| 52.9| 63.3| 100|    |    |    |
|      | 9 | 51.7| 52.3| 52.6| 44.6| 48.1| 55.9| 51.7| 48.7| 100|    |    |
|      | 10| 46.1| 41.5| 33.1| 47.6| 41.9| 45  | 43.7| 53.7| 53.5| 100|    |
|      | 11| 53.8| 51.2| 42.1| 47.6| 49  | 55.2| 54.7| 48 | 62 | 62.9| 100|
|      | 12| 50.1| 51.7| 35  | 50.9| 48.7| 50.9| 56.4| 46.8| 58.1| 51.5| 63.4| 100|
| C    |   |   |   |   |   |   |   |   |   |    |    |    |
| SP3  | 1 | 100|   |   |   |   |   |   |   |    |    |    |
|      | 2 | 64.2|   |   |   |   |   |   |   |    |    |    |
|      | 3 | 64.3| 72.7| 100|   |   |   |   |   |    |    |    |
|      | 4 | 62.6| 68 | 64.2| 100|   |   |   |   |    |    |    |
|      | 5 | 62.7| 55.1| 58.5| 56 | 100|   |   |   |    |    |    |
|      | 6 | 61.5| 64.8| 74.6| 63.8| 49.7| 100|   |   |    |    |    |
|      | 7 | 57.4| 35.4| 31.4| 34.4| 42.2| 36  | 100|   |    |    |    |
|      | 8 | 58  | 55.5| 55.5| 53.3| 64.1| 53.3| 49.6| 100|    |    |    |
|      | 9 | 51.4| 56.6| 61.2| 67.1| 56.5| 58.3| 41.2| 56 | 100|    |    |
|      | 10| 62.4| 68.4| 62.2| 63.3| 57.4| 66.3| 43.4| 72  | 65.7| 100|    |
|      | 11| 59.7| 58 | 61.9| 58.3| 61  | 66.5| 41.1| 48.8| 55.2| 55.9| 100|
|      | 12| 58.6| 47.9| 54.4| 43  | 47.6| 58.2| 33.1| 44.8| 45.9| 51  | 61.8| 100|

Table 2. Dice coefficient comparing the similarities of DGGE for soil eukaryotic micro-organisms in the SP1, SP2 and SP3 plots.
Figure 2. The UPGMA dendrogram generated using the DGGE profiles of samples collected from the SP1, SP2 and SP3 sampling sites.
The Shannon–Wiener index of the SP1 sampling site highest and lowest values were observed in the 5–15 cm soil layer in October and April (3.622 and 3.185). The value of the evenness index highest and lowest values were observed in the 15–25 cm soil layer in April and in the 0–5 cm topsoil layer in October (0.9953 and 0.9834). The richness index varied between 25–39 with an average value of 29. The lowest and highest values of Simpson’s diversity index were observed in the 5–15 cm soil layers in April and October (0.9575 and 0.9723). These results showed that the community structure of eukaryotic micro-organisms was affected by the sampling time and soil depth. Furthermore, the eukaryotic microbial community at the SP1 sampling site was diverse and displayed stable distribution.

The Shannon–Wiener diversity index of the SP2 sampling site highest and lowest values were observed at the 25–35 cm soil depth in April and in the 0–5 cm topsoil layer in October (3.504 and 2.769). The evenness index ranged from 0.9772–0.9905 with an average value of 0.9861. The richness index ranged from 17–35 with an average value of 24. The Simpson’s diversity index ranged from 0.9343–0.9688 with an average value of 0.9516. The highest and lowest values of this diversity index were observed in the 25–35 cm soil depth in April and in the 0–5 cm topsoil layer in October, respectively. These results showed that the community of eukaryotic micro-organisms present in the SP2 sampling site was relatively more diverse, abundant and homogeneous. Additionally, the diversity of the eukaryotic microbial community was dependent on the sampling time and soil depth.

The Shannon–Wiener diversity index of the soil eukaryotic micro-organisms in the sampling site SP3 highest and lowest values were observed in the 5–15 cm soil depth in October and in the 0–5 cm topsoil layer in April (3.418 and 3.067). The evenness index ranged from 0.9861–0.9924 with an average value of 0.9885. The richness index ranged from 22–32 with an average value of 25. The Simpson’s diversity index ranged from 0.9524–0.9657 with an average value of 0.9563. The results showed that the eukaryotic microbial community in the

### Table 3. The Shannon–Wiener, evenness, richness and Simpson indices of soil eukaryotic micro-organisms in the SP1, SP2 and SP3 plot.

| Time | Soil layer (cm) | Lane number | Shannon–Wiener (H) | Evenness (E) | Richness (S) | Simpson (D) |
|------|----------------|-------------|-------------------|--------------|--------------|-------------|
| April | 0–5            | 1           | 3.193             | 0.9920       | 25           | 0.9580      |
|       | 5–15           | 4           | 3.185             | 0.9895       | 25           | 0.9575      |
|       | 15–25          | 7           | 3.204             | 0.9953       | 25           | 0.9588      |
|       | 25–35          | 10          | 3.267             | 0.9914       | 27           | 0.9609      |
| July  | 0–5            | 2           | 3.249             | 0.9858       | 27           | 0.9598      |
| SP1   | 5–15           | 5           | 3.336             | 0.9908       | 29           | 0.9635      |
|       | 15–25          | 8           | 3.372             | 0.9914       | 30           | 0.9648      |
|       | 25–35          | 11          | 3.250             | 0.9862       | 27           | 0.9596      |
| October | 0–5         | 3           | 3.438             | 0.9834       | 33           | 0.9664      |
|       | 5–15           | 6           | 3.622             | 0.9886       | 39           | 0.9723      |
|       | 15–25          | 9           | 3.403             | 0.9911       | 31           | 0.9658      |
|       | 25–35          | 12          | 3.337             | 0.9909       | 29           | 0.9635      |
| April | 0–5            | 1           | 3.102             | 0.9892       | 23           | 0.9538      |
|       | 5–15           | 4           | 2.945             | 0.9830       | 20           | 0.9453      |
|       | 15–25          | 7           | 3.002             | 0.9861       | 25           | 0.9485      |
|       | 25–35          | 10          | 3.504             | 0.9856       | 35           | 0.9688      |
| July  | 0–5            | 2           | 3.023             | 0.9781       | 22           | 0.9490      |
| SP2   | 5–15           | 5           | 2.967             | 0.9905       | 20           | 0.9473      |
|       | 15–25          | 8           | 2.965             | 0.9896       | 20           | 0.9472      |
|       | 25–35          | 11          | 3.319             | 0.9857       | 29           | 0.9515      |
| October | 0–5          | 3           | 2.769             | 0.9772       | 17           | 0.9343      |
|       | 5–15           | 6           | 3.186             | 0.9897       | 25           | 0.9573      |
|       | 15–25          | 9           | 3.259             | 0.9889       | 27           | 0.9605      |
|       | 25–35          | 12          | 2.914             | 0.9898       | 19           | 0.9444      |
| April | 0–5            | 1           | 3.067             | 0.9924       | 22           | 0.9524      |
|       | 5–15           | 4           | 3.096             | 0.9873       | 23           | 0.9530      |
|       | 15–25          | 7           | 3.136             | 0.9868       | 24           | 0.9546      |
|       | 25–35          | 10          | 3.265             | 0.9906       | 27           | 0.9606      |
| July  | 0–5            | 2           | 3.147             | 0.9903       | 24           | 0.9558      |
| SP3   | 5–15           | 5           | 3.102             | 0.9894       | 23           | 0.9536      |
|       | 15–25          | 8           | 3.213             | 0.9861       | 26           | 0.9588      |
|       | 25–35          | 11          | 3.147             | 0.9901       | 24           | 0.9557      |
| October | 0–5         | 3           | 3.223             | 0.9891       | 26           | 0.9588      |
|       | 5–15           | 6           | 3.418             | 0.9862       | 32           | 0.9657      |
|       | 15–25          | 9           | 3.140             | 0.9882       | 24           | 0.9551      |
|       | 25–35          | 12          | 3.093             | 0.9865       | 23           | 0.9527      |

Note: SP1: Bird Island Protection Station in Jinhe’s Main Lake Inlet, SP2: meadow area of Bird Appreciate Station in Bole’s Main Lake Inlet, SP3: Salt-works of overflow area in Jinhe’s Main Lake Inlet.
SP3 sampling site of the Ebinur Lake wetland was diverse, with uniform distribution of microbes. Although the fluctuation in community diversity was not considerable, the community structure of eukaryotic microbes was affected by the sampling time and soil depth.

**Construction of phylogenetic tree using the 18S rDNA sequences of eukaryotic micro-organisms**

In order to further understand the diversity of eukaryotic microbial community in the Ebinur Lake wetland, the 36 bands obtained after performing DGGE were excised from the gel. The phylogenetic trees were constructed using the N-J method with 1000 boot, as shown in Figure 3. The unique organisms present in the SP1 and SP3 sampling sites were the fungi, while those present in the SP2 sampling site belonged to Alveolata. The community composition of eukaryotic micro-organisms present in the sampling sites SP1 and SP3 was similar. The composition of the eukaryotic microbial community in the Ebinur Lake wetland was observed to be different between the Jinghe and Bortala River basins. The results showed that the most dominant group of eukaryotic micro-organisms present in the Ebinur Lake wetland belonged to Viridiplantae, with 16 DNA sequences belonging to this group (which accounted for 44.44% of the total sequences). The second dominant group belonged to the division Metazoa with 14 sequences belonging to this group (which accounted for 38.89% of the total sequences). Together, these two groups accounted for 83.33% of total sequences. In addition, several unique species were observed at different sampling sites. In the sampling sites SP1 and SP3, fungal species were uniquely present. In the sampling site SP2, members belonging to clade Alveolata were uniquely present. The results showed that the eukaryotic microbial community of the Ebinur Lake wetland was rich. Additionally, the wetland also harboured a number of novel eukaryotic microbial species which need to be further investigated.

The similarities between the DNA sequences obtained after sequencing were analysed using BLAST. It was observed that the eukaryotic micro-organisms present in the Ebinur Lake wetland mainly belonged to clade Streptophyta in Viridiplantae; phylum Nematoda in Metazoa accounts for 50% of the taxon (four sequences of which belong to Chromadorea and three sequences belong to the genus Pristomatolaimus). There were four Chordata sequences accounting for 28.57% and three Arthropoda sequences accounting for 21.43% (including two sequences belonging to subphylum Crustacea and one sequence belonging to subphylum Hexapoda). Three sequences belonging to kingdom Fungi were observed, one of which belonged to family Leptosphaeriaceae of phylum Ascomycota, and the other two belonged to Alveolata (with one sequence belonging to phylum Ciliophora).

A preliminary study by Liu et al. [17] related to the molecular genetic diversity of phytoplanktons in the Jiaozhou Bay reported that the isolated organisms were phytoplanktons belonging to Cryptophyta, Bacillariophyta, Chlorophyta and Streptophyta. Further, few members (such as Rhododendron and original bacteria) were derived from the algal blooms. Duan et al. [18] studied the diversity of eukaryotic micro-organisms present in the sediments of abandoned coal wells in Yangbajing, Tibet. They observed that the major eukaryotic micro-organisms present in the sediments were ascomycetes, Listeria, basidiomycetes and uncultured fungi. Among them, Chytridiales sp., Physodermamaydis, Powellomyces sp., Candida parapsilosis, Zygosaccharomyces rouxii, Aureobasidium pullulans and Lecane bulla have not been reported from hot springs or hot wells. Broady et al. [19] studied the algae found in the Antarctic region. They reported 11 species of green algae (eukaryotes) and six species of cyanobacteria (prokaryotes). Takeuchi et al. [20] reported five species of green algae and cyanobacteria from the Akkrm glacier. In conclusion, we observed that the algal species present in the Ebinur Lake wetland were unique. These observations were consistent with the observations reported from the eukaryotic microbial communities in other environments, although some differences do exist. This may be due to the unique geographical location of the Ebinur Lake wetland and its high soil salinity, which potentially resulted from the process of the eukaryotic micro-organisms adapting to their environment.

These results also showed that the differential distribution of eukaryotic micro-organisms in the Ebinur Lake wetland was attributed to the differences in the soil environment conditions of the sampling sites. The unique micro-organisms present in the sampling sites SP1 and SP3 were the fungi and those present in the sampling site SP2 belonged to Alveolata. It was also demonstrated that the soil fertility of sampling sites SP1 and SP3 was similar and differed from the fertility of sampling site SP2. This was mainly because the sampling sites SP1 and SP3 were located in the Jinghe River basin, while the sampling site SP2 was located in the Bortala River basin. Considerable differences in the soil quality status of the sampling sites resulted in their respective eukaryotic microbial community structure.

The band SP2-6 (obtained from sample SP2) and the bands SP3-1, SP3-6 and SP3-8 (obtained from sample SP3) belonged to the chordates (phylum Chordata), which are closely associated with human activities. This
Figure 3. Phylogenetic tree constructed using the neighbour-joining method, based on the DGGE profiles of the samples collected from the SP1, SP2 and SP3 sampling sites.
indicated that anthropogenic activities had a considerable effect on the community structure and distribution of eukaryotic organisms in the Ebinur Lake wetland.

**Correlation of the eukaryotic microbial diversity indices with soil environment factors**

The SPSS software was used to analyse the diversity indices of eukaryotic micro-organisms and their correlation with the soil environment factors. The Shannon–Wiener diversity index of the soil eukaryotic micro-organisms showed a significant \( (P < 0.01) \) negative correlation with the organic matter and alkali-hydrolysable nitrogen contents of the soil (Table 4). The correlation coefficients were calculated to be 0.505 and 0.513, negatively correlated with the soil water and available potassium contents and positively correlated with the other soil environment factors; however, the correlation was not strong. A significant \( (P < 0.01) \) negative correlation was observed between the evenness index and the contents of organic matter, available nitrogen and available potassium (correlation coefficients were calculated to be 0.534, 0.546 and 0.506, respectively). The diversity index showed a negative correlation with the soil water content, conductivity, available phosphorus, chromium (Cr) and lead (Pb). The richness index showed a significant \( (0.01 < P < 0.05) \) negative correlation with the organic matter and alkali-hydrolysable nitrogen contents. The correlation coefficients were calculated to be 0.421 and 0.412, respectively. The diversity index showed a negative correlation with the soil water content and available potassium and a positive correlation with the other soil environment factors. The Simpson’s diversity index showed significant \( (P < 0.01) \) negative correlation with the contents of organic matter and alkali-hydrolysable nitrogen \( (r = 0.545 \text{ and } r = 0.562, \text{ respectively}) \). At the same time, the diversity index showed a positive correlation with the other soil environment factors, but it showed a negative correlation with the soil water content and available potassium. In summary, the soil environment factors affecting the eukaryotic microbial community structure in the Ebinur Lake wetland showed a positive correlation with the contents of organic matter, available nitrogen and available phosphorus; however, they showed a negative correlation with the other environmental factors. The major soil environment factor affecting the eukaryotic microbial diversity in the Ebinur Lake wetland was soil fertility. The higher the soil fertility, the lower the diversity of eukaryotic microbes. In other words, the poorer the quality of soil, the higher the microbial diversity. Therefore, in this study, since the soil eukaryotic micro-organisms displayed high biodiversity, it reflected that the soil nutrients present in the Ebinur Lake wetland were low and the soil was barren.

Jin et al. [21] reported that the soil organic carbon, total nitrogen, total phosphorus and available phosphorus contents showed a significant negative correlation with the Shannon–diversity index, which was associated with the soil fertility with prolongation of enclosure time. The higher the soil fertility, the lower the level of soil eukaryotic diversity. Ellis et al. [22] observed that the heavy metal content specifically affected the physiological characteristics of soil micro-organisms.

**Table 4.** Correlation analysis between the soil eukaryotic micro-organisms diversity indices and environmental factors.

| Environmental factors | Correlation | Shannon index (H) | Evenness index (E) | Abundance index (S) | Simpson index (D) |
|-----------------------|-------------|-------------------|-------------------|--------------------|------------------|
| SM                    | r           | 0.22              | -0.204            | -0.161             | -0.223           |
|                       | p           | 0.198             | 0.232             | 0.348              | 0.191            |
| PH value              | r           | 0.274             | 0.322             | 0.19               | 0.327            |
|                       | p           | 0.106             | 0.056             | 0.266              | 0.051            |
| EC                    | r           | 0.079             | -0.088            | 0.043              | 0.083            |
|                       | p           | 0.647             | 0.61              | 0.804              | 0.623            |
| OM                    | r           | -0.505**          | -0.534**          | -0.421*            | -0.545**         |
|                       | p           | 0.002             | 0.001             | 0.011              | 0.001            |
| AN                    | r           | -0.513**          | -0.546**          | -0.412*            | -0.562**         |
|                       | p           | 0.001             | 0.001             | 0.013              | 0.0001           |
| AP                    | r           | 0.069             | -0.114            | 0.106              | 0.036            |
|                       | p           | 0.688             | 0.508             | 0.539              | 0.833            |
| AK                    | r           | -0.072            | -0.306**          | -0.034             | -0.117           |
|                       | p           | 0.676             | 0.002             | 0.843              | 0.496            |
| Cr                    | r           | 0.121             | -0.017            | 0.117              | 0.122            |
|                       | p           | 0.482             | 0.921             | 0.496              | 0.479            |
| Cu                    | r           | 0.060             | 0.010             | 0.050              | 0.068            |
|                       | p           | 0.728             | 0.953             | 0.771              | 0.693            |
| Pb                    | r           | 0.274             | -0.086            | 0.287              | 0.266            |
|                       | p           | 0.106             | 0.619             | 0.090              | 0.117            |
| Zn                    | r           | 0.189             | 0.021             | 0.203              | 0.118            |
|                       | p           | 0.270             | 0.901             | 0.234              | 0.271            |

Note: SM, soil moisture; EC, electrical conductivity; OM, organic matter; AN, Alkaline nitrogen; AP, available phosphorus; AK, available potassium; Cd, cadmium; Cr, chromium; Cu, copper; Pb, lead; Zn, zinc.

*Correlation is significant \( (0.01 < P < 0.05) \).

**Correlation is very significant below the level of 0.01 \( (P < 0.01) \).
Analysis of the correlation between soil environmental factors and eukaryotic microbial community composition in the Ebinur Lake wetland showed that the major soil environmental factors influencing the eukaryotic microbial community composition were soil water content, pH, salinity, nutrient content and heavy metals. Griffiths et al. [23] studied the effects of water availability on microbial diversity in the steppes and found that water could regulate microbial diversity and structure. Kowalchuk et al. [24] found that pH was the main factor affecting the diversity of Nitrosospira sp. Sun et al. [25] found that the long-term application of organic manure could efficiently maintain the original structure of the soil microbial community. Lorenz et al. [26] studied results showed that the microbial communities isolated from the soils contaminated with these two heavy metals were considerably different. However, the endurance of fungi and proteobacteria towards these two heavy metals in the soil was relatively strong. As a result, the richness of these two species remained unchanged. This indicated that the heavy metal content of the soil, which had been a cause of concern for a long time, had considerably less effect on the diversity of soil eukaryotic micro-organisms.

Conclusions

The diversity indices calculated for the three sampling sites in the Ebinur Lake wetland using DGGE showed that the diversity of soil eukaryotic micro-organisms in the Jinghe River basin was higher compared to that in the Bortala River basin. This indicated the dynamic succession of eukaryotic microbes in the Ebinur Lake wetland and the changes in the dominant microbial composition. Algae were the main group of eukaryotic microbes present in the Ebinur Lake wetland, but the species composition of algae in Ebinur Lake was relatively simple. This may be due to the unique geographical location of the Ebinur Lake wetland and its high soil salinity, which potentially resulted from the process of the eukaryotic micro-organisms adapting to their environment. The major soil environment factors affecting the structure of eukaryotic microbial communities in the Ebinur Lake wetland were soil water content, pH, salinity, nutrient content and heavy metal ions. However, the heavy metal content, which had earlier attracted much attention, had considerably less effect on the diversity of soil eukaryotic micro-organisms.

Geolocation information

The Ebinur Lake wetland is located in the north-eastern part of Bole city in Xinjiang Uyghur Autonomous Region (Longitude, 82° 36′–83° 50′ East; Latitude, 44° 37′–45° 15′ North) of China.

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Disclosure statement

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