Assessment of Microbial Diversity of *Deyeuxia angustifolia* Wetland through Phospholipid Fatty Acids (PLFA) in Sanjiang Plain

Jifeng Wang¹, Mengsha Li², Tong Zhang², Xin Sui²,³, Weichao Ma² and Hong-Wei Ni³

¹College of Geographical Science, Harbin Normal University, Harbin 150040, China
²College of Life Science, Heilongjiang University, Harbin 150080, China
³Institute of Nature & Ecology, Heilongjiang Academy of Sciences, Harbin 150040, China

Abstract

The Sanjiang plain is the most important wetland ecosystem in northeast China. Due to human activity, the water level is severely lowered, resulting in various stages of wetland degradation, with subsequent changes of ecosystem functions. Here we studied the changes in microbial content and diversity of soil in marsh, marsh meadow and meadow wetland, each of these is typically covered by *Deyeuxia angustifolia*. The method applied was qualitative and quantitative analysis of phospholipid fatty acids (PLFA). 72 types of PLFA were detected in the soil types, of which 29 could be attributed to microbial groups, with prokaryotes being most often detected. The detected characterized PLFA represented a soil microbial biomass of approximately 2 to 31 nmol·g⁻¹ dry soil. Compared to soil from marsh wetland, the total content of PLFA, and PLFA derived from Gram-positive bacteria and Gram-negative bacteria was higher in meadow and marsh meadow soil. The fatty acid content in bacteria, fungi and protozoa varied between the soil types with lower values in marsh wetland than the other soil types. Canonical correlation analysis identified that soil pH, water content and total nitrogen content were the most influential factors determining the amount of bacteria in soil, while the nitrate nitrogen content also had an impact. The microbial community structure became more diverse as wetland degradation progressed. © 2018 Friends Science Publishers

Keywords: Nitrogen deposition; Phospholipid fatty acids; *Deyeuxia angustifolia* wetland; Microbial diversity

Introduction

Wetlands, display the richest biodiversity and highest productivity amongst all ecosystems in nature, are important for the reduction of greenhouse gas emission, conservation of biodiversity, and contribute to regulating the balance of the earth’s ecosystem (Galloway et al., 2004; Dentener et al., 2006; Andersen et al., 2010). The wetland of Sanjiang plain, in northeast China, is the largest freshwater wetland of the country, and with its richness in animals and plant species, it displays a high biodiversity. Its role in maintaining the ecological balance in the region is irreplaceable. However, in recent decades, large-scale agricultural activities in Sanjiang plain have led to loss of vast areas of wetland, reducing wetland coverage from 80% in 1950s to only 20% at present (Sui et al., 2015). Due to human activity, original wetland, typically covered with *Deyeuxia angustifolia*, degraded to marsh meadow and further to typical meadow land, a transition that has been subject to many investigations (Lu et al., 2007).

Soil microbes are mainly responsible for decomposition of organic matter. These participate in the biogeochemical processes that recycle soil elements, and play a crucial role in maintaining these elements in an ecosystem (Wieten et al., 2012). The structure of soil microbe populations is dictated by plant diversity as well as by physical and chemical properties of the soil. Although *D. angustifolia* dominance was maintained in wetland under going different degradation stages, the soil environment demonstrated significant changes during the transition from pristine wetland to marsh meadow and further to meadow land. It is crucial to understand how these microbial population structures and compositions change, being the basis of any ecosystem.

In recent years, novel methods and techniques have been applied to study soil microbiology, such as denaturing gradient gel electrophoresis (DGGE) (Anderson et al., 2010), phospholipid fatty acids (PLFA) analysis (Wright et al., 1995), and microbial environmental whole genome sequencing (Kazda, 1990). PFLA are the main constituents of microbial cell membranes, and disintegrate rapidly after cell death. Since their composition widely varies between microbial species, qualitative and quantitative analysis of PFLA provides a good measure of living microbial populations (Lovett et al., 1982). By analyzing the type and composition ratio of PFLAs, the precise microbial community structure and diversity can objectively and quantitatively be described. Here we analyzed soil microbial...
diversity by PFLA detection using three types of *D. angustifolia* wetlands from Sanjiang plain: pristine wetland, marsh meadow wetland, and meadow wetland. This research provides a scientific basis for the change of the soil ecosystem in Sanjiang plain and offers a theoretical reference for protection and management of wetlands in this area.

**Materials and Methods**

**Description of Studied Area**

Three *Deyeuxia angustifolia* wetlands at different degradation stages in Sanjiang plain were selected for this study. These were located at three research stations in Honghe National Nature Reserve, the institute of Natural and Ecology, Heilongjiang Academy of Sciences, China. The research stations are located at an altitude of 50 to 65 m, where the annual mean temperature is 1.9°C, and the effective accumulated temperature (≥10°C) is 2165 ~ 2624°C. The annual average precipitation is 585 mm, and 50% to 70% of the precipitation occurs between June and September. The annual average evaporation is 1166 mm. The sampling wetlands reflected the three states of pristine wetland, meadow marsh wetland and meadow wetland, with physico-chemical soil characteristics described before. A table summarizing these is available as supplementary information. Vegetation characteristics are shown in Table 1.

**Sample Collection**

Using a random sampling method, three 10 m × 10 m sample plots were selected in each wetland type, separated by a distance of 50 m. Soil samples were collected in August, 2015 at a depth of 0 to 20 cm by a soil drill with a diameter of 4 cm. A collection of samples taken at 5 – 10 collection points per plot was mixed. Debris such as stones and plant roots were removed and the soil was mixed uniformly before it was divided into four aliquots and transferred to lab in plastic bags in an icebox. One part was kept at 4°C for analysis of the diversity of the soil microbial community. The other part was air-dried, grinded, and passed through a 100-mesh sieve for determination of soil physical and chemical properties (Sui et al., 2017). The third part was used for extraction of PLFA.

**Phospholipid Fatty Acids Measurement**

The phospholipid fatty acids were extracted directly from soil as previously described (Drenovsky et al., 2004). Briefly, 3 g of soil sample was added to 38 mL extraction solution containing 3.8 mL of chloroform, 7.6 mL of 100% methanol, and 2.64 mL of 5 mM citric acid, pH 3. After mixing for 20 min the mixture was centrifuged and the chloroform supernatant was collected. Extraction was repeated and the two supernatants were combined. Citric acid was added to the supernatant, after which PFLA were extracted with 100% chloroform. After collection, the chloroform phase was dried using high-purity N2, redissolved in n-hexane and transferred to a silica gel column. The column was washed first with 100% chloroform, then with 100% acetone, and subsequently eluted with 100% methanol. The methanol eluate was collected and dried with high-purity N2. The precipitation was redissolved in 1 mL 1:1 methanol: toluene mixture (v/v) and 1 mL of 0.2 mol/L KOH in 100% methanol was added. After mixing this was incubated at 37°C for 20 min, cooled down to room temperature, and neutralized by addition of acetic acid. Next, 2 mL of chloroform:N-hexane (1:4 v/v) was taken and distilled water was added to a final volume. After mixing, the extraction solution was allowed to separate from the water phase, collected and dried by high-purity N2. Using the nineteen-alkane acid as internal marker, the composition of fatty acids was determined by gas chromatography (Agilent 6890). The concentration of each fatty acid was calculated by the method of Wang et al. (2008). Where the fatty acid data were converted to concentration (nmol/g dry soil) using the equation:

\[ C(x) = (A:B)xCxF \]

Where C(x) is the concentration of fatty acid x in nmol/g soil, A is the response value of the sample for the methyl ester of fatty acid x, B is the response of the internal marker C19:0, C is the concentration of C19:0 (ng/μL), V is the volume of the sample in μL, and FAME is the molecular mass of the fatty acid methyl ester of x (g/mol).

Data were further processed by Excel and plotted by Sigma Plot. SPSS19.0 software was used to conduct single factor analysis of variance (significance level of α=0.05), abundance (S), uniformity (J), and diversity index (Shannon-Wiener index (H) and Simpson index (D)), and canonical correlation analysis.
Results

PFLA are Biomarkers for Groups of Microorganisms

PLFA data can be used as a biomarker for a variety of microorganisms, as the composition of phospholipid fatty acids reflects the composition of total microbial biomass. Moreover, bacterial content can be divided into Gram-positive and Gram-negative bacterial content or into aerobes or anaerobes, while fungi and other microorganisms also result in specific PFLA results (Table 2). For some biomarkers, the organisms could be narrowed down for specific taxonomic groups, as indicated. The nomenclature introduced in that table is also used in the rest of the text (Table 2).

PFLA Presence in Soil from Wetlands of Different Degradation Stages

In this study, 72 different PLFA were detected from soil of three Deyeuxia angustifolia wetland types represent different stages of degradation stages. Of the detected PFLA, 29 could be attributed to microbe types, together with the detected concentrations in the three different soil types analyzed (Table 3). The carbon chain length varied from 11 to 24, including saturated, unsaturated, methylated branched and cyclic fatty acids.

Notable differences were detected between different soil types. In particular, soil from the least degraded wetland (marsh wetland) contained fewer PFLA. This is more obvious from Fig. 1, where the data shown in Table 3 were used to calculate the total microbial biomass that could be characterized by total PLFA content. This total ranged from 2 to 31 nmol/g, with a significantly lower finding for marsh wetland (P<0.05) (Fig. 1A).

Presence of Gram-negative bacteria inferred from PLFA data suggested a significantly (P<0.01) higher abundance in marsh and marsh meadow wetland soil than in meadow soil (Fig. 1C). However, variation in detected Gram-positive bacteria was not significant (Fig. 1B).

Variation of Bacteria, Fungi, and Actinomycetes Detected by PLFA

The total amount of detectable bacteria and fungi in the three soil types were used (Table 3). As shown in panel A of Fig. 2, the detectable bacterial content in soil of marsh wetland was significantly lower than the other two soil types (P < 0.05). Compared to marsh wetland, the bacterial content was increased by a factor of 4.6 in marsh meadow soil.

Abundance of detectable fungi is shown in Fig. 2B; again, a significantly lower content was detected in soil from marsh wetland (P < 0.01). Compared to soil type, the fungi content was increased by a factor of 5.69 in marsh meadow wetland.

Fig. 1: Total PLFA Content of Soil Microorganisms in Deyeuxia angustifolia wetlands at different degradation stages. Panel A: total microbial content; PanelB: Gram-positive bacteria; Panel C: Gram-negative bacteria. Significance is shown as * for P<0.05, ** for P<0.01

Finally, the Actinomycetes shown in Fig. 2C showed the same trend as that of bacteria and fungi. This group of bacteria displayed the largest difference between the three soil types, with an increase by a factor of 37.5 between marsh meadow compared to marsh Deyeuxia angustifolia wetland.

Variation in Soil Microbial Community Structure of three Different Soil Types

Three different diversity indices were calculated to express the microbial community structure, based on the PLFA data (Table 4).
Table 1: General properties of the studied areas

<table>
<thead>
<tr>
<th>Wetland Type</th>
<th>Location</th>
<th>Main plant types</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meadow Deyeuxia angustifolia</td>
<td>47°4′27″N, 133°3′51″E</td>
<td>D. angustifolia, Stellaria raduan, Anemone dichotoma, Lathyrus quinquenervius, Thalictrum simplex</td>
</tr>
<tr>
<td>Marsh meadow wetland (w1)</td>
<td>47°4′53.9″N, 133°3′70.4″E</td>
<td>D. angustifolia, Carexappendiculata, Lathyrus quinquenervius, Carex. myahbeivae. Maoppengensis</td>
</tr>
<tr>
<td>Marsh wetland (w2)</td>
<td>47°4′71.6″N, 133°3′74.3″E</td>
<td>D. angustifolia, Carex pseudo-comicon, C. myahbeivarmasopengensis, Glyceria spiculosa, Calamagrostis neglecta, Salix rosmarinifolia, Salix myrtilloides</td>
</tr>
</tbody>
</table>

Table 2: PLFA characteristics of microbial groups

<table>
<thead>
<tr>
<th>Microbial group</th>
<th>Phospholipids fatty acid biomarkers*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Saturated or monounsaturated fatty acid with an ester chain linked to glycerol, e.g., 15:0, 15:1, 15:2, 15:4, 15:5, 16:0, 16:1ω7t, 16:1ω5t, 16:1ω9t, 17:0ω7t, 17:0ω5t, 17:0ω9t, 18:1ω7t, 18:1ω5t, 18:1ω9t, 18:2ω7t, 18:2ω9t, 19:0ω and cy19:0</td>
</tr>
<tr>
<td>Acreobes</td>
<td>i13:0, i15:0, i15:5, i15:6, 16:1ω6t, 16:1ω7t, i17:0, a17:0, cy17:0, 18:1ω5t, 18:1ω7t, 18:1ω9t, 19:0ω and cy19:0</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>cy17:0, cy19:0</td>
</tr>
<tr>
<td>Gram-positive bacteria</td>
<td>Multiple branched fatty acids (iso—anteiso—e.g., 18:1 w7c 11 – methyl, 16:0 iso, 15:0 iso, 15:0 anteiso, 12:0, 11:0, 10:0 OH, 18:0 iso, 17:0 anteiso, 16:0 anteiso, 10 – methyl, 18:0 10 – methyl, TBSAm 18:1 w7c 11 – methyl, 17:0 iso</td>
</tr>
<tr>
<td>Gram-negative bacteria</td>
<td>Variety of hydroxy fatty acids (mono fatty acids, cyclic fatty acids etc.) e.g.16:1 w5c, 14:1 w5c, 16:0, 18:0, 13:0 iso, 17:1 w5c</td>
</tr>
<tr>
<td>Methane-oxidizing bacteria</td>
<td>16:1 w5c</td>
</tr>
<tr>
<td>Fungi</td>
<td>Containциque phospholipid fatty acids, e.g., 18:1 w6t, 18:2 w6t, 18:3 w6t, 18:3 w3</td>
</tr>
<tr>
<td>Protozoa</td>
<td>20:3 w5c, 20:4 w5c</td>
</tr>
<tr>
<td>Bacterial taxonomic group</td>
<td></td>
</tr>
<tr>
<td>Actinomycetes</td>
<td>10Me16:0, 10Me17:0, 10Me18:0, 17:0 10 – methyl, 18:0 10 – methyl, TBSA</td>
</tr>
<tr>
<td>Pseudomonas sp.</td>
<td>14:1 w5c, 16:0, 17:0</td>
</tr>
<tr>
<td>Arthrobacter sp.</td>
<td>17:0</td>
</tr>
<tr>
<td>Cellulomonas sp.</td>
<td>18:1 w7c 11 – methyl</td>
</tr>
<tr>
<td>Flavobacterium sp.</td>
<td>13:0 iso</td>
</tr>
</tbody>
</table>

*iso—anteiso: cyclopropyl and Mc-methyl branching fatty acids. o—aliphatic and cis configuration; t—trans configuration

We summarize the type of fatty acids that can be used as unique biomarkers for groups of organisms, as compiled from the literature (Liu et al., 2007; Cai and Zhang, 2008; Lei et al., 2014)

Table 3: PLFA biomarkers detected in soil of three different Deyeuxia angustifolia wetlands

<table>
<thead>
<tr>
<th>PLFA name</th>
<th>Microbial types</th>
<th>Meadow wetland (nmol/g)</th>
<th>Marsh meadow wetland (nmol/g)</th>
<th>Pristine wetland (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:00</td>
<td>Bacteria</td>
<td>0.1442</td>
<td>0.0386</td>
<td>0</td>
</tr>
<tr>
<td>14:00</td>
<td>Bacteria</td>
<td>0.4880</td>
<td>0.5182</td>
<td>0.0296</td>
</tr>
<tr>
<td>15:1ωc</td>
<td>Bacteria</td>
<td>0.1855</td>
<td>0.1806</td>
<td>0.0251</td>
</tr>
<tr>
<td>15:00</td>
<td>Bacteria</td>
<td>0.8536</td>
<td>0.4097</td>
<td>0.0228</td>
</tr>
<tr>
<td>19:0 iso</td>
<td>Bacteria</td>
<td>0.8516</td>
<td>0.0539</td>
<td>0</td>
</tr>
<tr>
<td>20:00</td>
<td>Bacteria</td>
<td>0.3226</td>
<td>0.3240</td>
<td>0.2706</td>
</tr>
<tr>
<td>16:0 iso</td>
<td>Gram-positives</td>
<td>1.5322</td>
<td>1.7973</td>
<td>0.0517</td>
</tr>
<tr>
<td>15:0 iso</td>
<td>Gram-positives</td>
<td>3.7914</td>
<td>3.5318</td>
<td>0.1356</td>
</tr>
<tr>
<td>15:0 anteiso</td>
<td>Gram-positives</td>
<td>3.7424</td>
<td>3.3489</td>
<td>0.1350</td>
</tr>
<tr>
<td>11:0 3 OH</td>
<td>Gram-positives</td>
<td>0</td>
<td>0.0263</td>
<td>0</td>
</tr>
<tr>
<td>18:0 iso</td>
<td>Gram-positives</td>
<td>0.1984</td>
<td>0.2149</td>
<td>0.0309</td>
</tr>
<tr>
<td>17:0 anteiso</td>
<td>Gram-positives</td>
<td>1.1829</td>
<td>1.1503</td>
<td>0.0968</td>
</tr>
<tr>
<td>16:0 anteiso</td>
<td>Gram-positives</td>
<td>0.1426</td>
<td>0.1630</td>
<td>0.0366</td>
</tr>
<tr>
<td>17:1 w8c</td>
<td>Gram-positives</td>
<td>0.4417</td>
<td>0.5534</td>
<td>0.0282</td>
</tr>
<tr>
<td>17:0 iso</td>
<td>Gram-positives</td>
<td>1.0836</td>
<td>1.0333</td>
<td>0.0633</td>
</tr>
<tr>
<td>14:0 iso</td>
<td>Aerobes</td>
<td>0.2674</td>
<td>0.2800</td>
<td>0</td>
</tr>
<tr>
<td>16:1 w5c</td>
<td>Methane-oxidizing Gram-positives</td>
<td>1.2031</td>
<td>0.9964</td>
<td>0.0528</td>
</tr>
<tr>
<td>13:0 w6c</td>
<td>Flavobacterium sp.</td>
<td>0.0316</td>
<td>0.0567</td>
<td>0</td>
</tr>
<tr>
<td>14:1 w5c</td>
<td>Pseudomonas sp.</td>
<td>0.0402</td>
<td>0.0442</td>
<td>0</td>
</tr>
<tr>
<td>16:00</td>
<td>Pseudomonas sp.</td>
<td>5.7186</td>
<td>5.4822</td>
<td>0.4144</td>
</tr>
<tr>
<td>17:00</td>
<td>Actrobacter sp.</td>
<td>0.4472</td>
<td>0.4341</td>
<td>0.0276</td>
</tr>
<tr>
<td>17:0 10 – methyl</td>
<td>Actinomycetes</td>
<td>0.5808</td>
<td>0.7377</td>
<td>0.0160</td>
</tr>
<tr>
<td>18:00</td>
<td>Hydrogenobacter</td>
<td>1.3893</td>
<td>1.4376</td>
<td>0.1122</td>
</tr>
<tr>
<td>18:1 w7c 11 – methyl</td>
<td>Cellulomonas sp.</td>
<td>0.5007</td>
<td>0.4890</td>
<td>0.0483</td>
</tr>
<tr>
<td>18:0 10 – methyl, TBSA</td>
<td>Actinomycetes</td>
<td>1.3887</td>
<td>1.5168</td>
<td>0.0483</td>
</tr>
<tr>
<td>18:3 w6c (6:9,12)</td>
<td>Fungi</td>
<td>0.2284</td>
<td>0.1571</td>
<td>0.0258</td>
</tr>
<tr>
<td>18:1 w9c</td>
<td>Fungi</td>
<td>4.9888</td>
<td>6.0597</td>
<td>0.1128</td>
</tr>
<tr>
<td>20:4 w6,9,12,15c</td>
<td>Protozoa</td>
<td>0.2457</td>
<td>0</td>
<td>0.1647</td>
</tr>
<tr>
<td>20:2 w6,8c</td>
<td>Protozoa</td>
<td>0.1788</td>
<td>0.1564</td>
<td>0.3099</td>
</tr>
</tbody>
</table>

Table 4: Diversity indices of PLFA in Deyeuxia angustifolia wetland soil at different degeneration stages

<table>
<thead>
<tr>
<th>Wetland type</th>
<th>Pielou uniformity index*</th>
<th>Shannon-Weiner index*</th>
<th>Simpson index*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meadow Deyeuxia angustifolia</td>
<td>0.79±0.17</td>
<td>2.63±0.17</td>
<td>0.90±0.01</td>
</tr>
<tr>
<td>Marsh meadow Deyeuxia angustifolia</td>
<td>0.78±0.18</td>
<td>2.60±0.18</td>
<td>0.89±0.00</td>
</tr>
<tr>
<td>Marsh Deyeuxia angustifolia</td>
<td>0.87±0.29</td>
<td>2.71±0.21</td>
<td>0.91±0.00</td>
</tr>
</tbody>
</table>

Different superscripts indicate statistically significant (P<0.05) differences between entries within a column for a given index
The detected fatty acids suggest differences in presence of bacteria, fungi, *Actinomycetes*, and protoza between the soil types, resulting in different diversity indices (Table 4). The marsh wetland has the highest soil microbial Shannon-Weiner (2.71) and Pielou uniformity (0.87) index. The other two soil types are comparable to each other. From this it is concluded that the original marsh wetland has the highest microbial diversity which is reduced as the degradation sets in. The Simpson indices of all three soil types were not statistically different.

To further study the influence of environmental factors on microbial community structure, SPSS was employed to conduct canonical correlation analysis.

The main factor of $V_1$ are pH and water content in the soil, while for $U_3$ the pH and total nitrogen content are the dominant factors. However, the effect of pH is opposite for these two variables.

The canonical variables of microbial community structure factors include bacterial content (BC), fungi content (FC), *actinomycetes* content (AC) and total microbial content (TM) (Table 7). That analysis resulted in the following correlations for the first two variables:

$$U_1 = 6.576[pH]–0.234[OC]–0.836[TN]+0.784[AN]–0.949[NN]–2.437[WC].$$

$$U_3 = -0.234[pH]–0.234[OC]+0.705[TN]+0.474[AN]–0.498[NN].$$

Thus, the main influence factor for $U_1$ are pH and water content in the soil, while for $U_3$ the pH and total nitrogen content are the dominant factors. However, the effect of pH is opposite for these two variables.

The canonical variable correlation coefficient and significance test

<table>
<thead>
<tr>
<th>Group</th>
<th>Wilk’s Chi-Sq</th>
<th>DF</th>
<th>Sig</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>36,000</td>
<td>.000</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>148.246</td>
<td>25,000</td>
<td>.000</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>95.221</td>
<td>16,000</td>
<td>.000</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>45,003</td>
<td>9,000</td>
<td>.000</td>
</tr>
<tr>
<td>5</td>
<td>.686</td>
<td>.565</td>
<td>4</td>
<td>.967</td>
</tr>
<tr>
<td>6</td>
<td>.979</td>
<td>.032</td>
<td>1</td>
<td>.967</td>
</tr>
</tbody>
</table>

Here $U$ represents standardized environmental variables, and $V$ represents standardized variables for biomass of community structures. As shown in Table 5, a Bartlett $\chi^2$ measurement was conducted on $U$ and $V$, and six groups of correlation coefficients were obtained. According to the Sig. value, as $\alpha = 0.05$, the top 4 canonical correlation coefficients are extremely significant with $r > 0.5$.

**Soil Characteristics Define the Microbial Structure of Wetland Soil**

An attempt was made to correlate the microbial findings to particular soil characteristics. Important physico-chemical properties of the three soil types are summarized in Table 6. There is a general trend in the order of meadow soil to marsh meadow to meadow soil, with progressively more water, lower pH, and a decrease in total organic carbon (OC), total nitrogen content (TN), and nitrate and ammonium nitrogen content (AN and NN, respectively). These differences are statistically significant. Thus, the increase of water content reduces the nutrient content in soil.

A canonical component analysis was first carried out to identify correlations between these physico-chemical properties, with results shown in Table 7. The calculations resulted in the following correlations for the first two variables:

$$V_1 = 1.744[TM]–28.130[BC]–67.958[FC]+124.470[AC].$$

$$V_2 = -0.398[TM]+0.647[BC]–0.660[FC]–1.327[AC].$$

The main factor of $V_1$ and $V_2$ is the content of *Actinomycetes* but its effect is opposite in these two variables.
According to the canonical correlation analysis for U and V, the soil pH, water content, and total nitrogen content have significant influence on Actinomycetes content in the microbial community. It can be concluded that wetland degradation creates the largest impact on Actinomycetes in the soil microbial community, followed by effects on fungi and bacteria (Table 8).

### Discussion

#### Influence of Wetland Degradation on Soil Microbial Biomass

Wetland degradation can lead to changes of soil microbial biomass and community structures, and as shown here, such changes can be identified by PLFA analysis. As wetland changed from marsh *Deyeuxia angustifolia* wetland to marsh meadow and finally to meadow wetland, the detectable total biomass of bacteria, fungi and other microbes in soil had greatly increased of Sanjiang plain (Fig. 1 and 2). Marsh wetland is flooded throughout the year, leading to poor soil aeration and low content of DOC, MBC, and EOC in soil (Liu et al., 2007), which is unfavorable for soil microbes. As wetland degrade, not only water content but also other physical and chemical properties of soil undergo changes. The nutrient content of the surface layer of the soils increases, which supports more soil microbes, of more diverse species (Table 3). Other studies have also shown changes in soil microbial biomass due to degradation in various ecosystems. For example, Lei et al. (2014) studied soil microbes in alpine meadow soil from the region of Sanjiang at different degradation successions, and reported that the soil microbial biomass reached a maximum during the middle degradation stage. Similarly, Cai and Zhang (2008) found that the activity and content of soil microbes in alpine grassland soil at different degradation stages reached a maximum at a certain degree of disturbance. The study showed that the soil microbial biomass of marsh meadow wetland is significantly higher than the other two investigated types, probably as a result of increased abundance of nutrient resources, which enhanced population complexity, maintaining the stability of the community. Thus, the community productivity is enhanced, increasing the amount of decomposed plant litter and soil fertility (Kazda, 1990).

It is found that the soil pH, soil water content, and total nitrogen content all influence the microbial content. It has been shown earlier that soil microbial content in wetlands is affected by soil water content, pH, availability of nutrients, plant types, etc. For example, Zhao and Zhou (2006) studied the characteristics of soil microbial content in coastal reed wetland soil from Panjin, China, and found that changes in the content of bacteria and total microbes was mainly affected by changes in soil water content. In their study, the fungi content was influential by a synergistic effect of soil water and temperature. The same ecosystem was studied by Liu et al. (2007) who found that the soil microbial content in coastal reed wetland was affected by soil pH, nutrients, and plant type. Using an experimental approach, Gong et al. (2015) found that soil water content and plant types were the main factors responsible for a changed soil microbial community. The soil investigated in this work is mainly covered by *D. angustifolia*. Thus, differences in plant type are not the representative for the observed differences in soil

### Table 6: Physical and chemical properties of the three different soil types

<table>
<thead>
<tr>
<th>Wetland type</th>
<th>Water content pH (WC) (%)</th>
<th>Organic content (g/kg)</th>
<th>Total carbon content (OC) (g/kg)</th>
<th>Total nitrogen content (TN) (mg/kg)</th>
<th>Ammonium nitrogen content (AN) (mg/kg)</th>
<th>Nitrate nitrogen content (NN) (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meadow <em>Deyeuxia angustifolia</em> wetland</td>
<td>75±0.10 * a</td>
<td>5.82±0.01 *</td>
<td>47.91±0.16 *</td>
<td>2.88±0.02 a</td>
<td>20.17±0.56 a</td>
<td>5.15±0.05 a</td>
</tr>
<tr>
<td>Marsh meadow <em>Deyeuxia angustifolia</em> wetland</td>
<td>86±0.08 * a</td>
<td>5.66±0.02 b</td>
<td>44.23±0.19 b</td>
<td>2.70±0.02 b</td>
<td>18.51±0.56 b</td>
<td>4.41±0.08 b</td>
</tr>
<tr>
<td>Marsh <em>Deyeuxia angustifolia</em> wetland</td>
<td>185±0.11 * c</td>
<td>5.56±0.01 *</td>
<td>42.32±0.12 *</td>
<td>2.27±0.01 *</td>
<td>7.47±0.56 a</td>
<td>4.25±0.07 a</td>
</tr>
</tbody>
</table>

Different superscripts indicate statistically significant (P<0.05) differences between entries within a column

### Table 7: Correlation coefficients for canonical variables U and soil physico-chemical variables

<table>
<thead>
<tr>
<th>Factor*</th>
<th>U1</th>
<th>U2</th>
<th>U3</th>
<th>U4</th>
<th>U5</th>
<th>U6</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>6.576</td>
<td>-0.234</td>
<td>0.836</td>
<td>0.949</td>
<td>0.784</td>
<td>2.437</td>
</tr>
<tr>
<td>Organic carbon (OC)</td>
<td>-0.234</td>
<td>1.351</td>
<td>1.18</td>
<td>8.778</td>
<td>6.25</td>
<td>1.171</td>
</tr>
<tr>
<td>Total nitrogen (TN)</td>
<td>-0.836</td>
<td>0.705</td>
<td>2.314</td>
<td>3.828</td>
<td>2.314</td>
<td>3.828</td>
</tr>
<tr>
<td>Nitrate nitrogen (NN)</td>
<td>-0.949</td>
<td>-0.498</td>
<td>-0.600</td>
<td>-8.483</td>
<td>-1.355</td>
<td>-11.75</td>
</tr>
<tr>
<td>Ammonium nitrogen (AN)</td>
<td>0.784</td>
<td>0.474</td>
<td>0.830</td>
<td>13.615</td>
<td>-4.838</td>
<td>-0.814</td>
</tr>
<tr>
<td>Soil water content (WC)</td>
<td>-2.437</td>
<td>-0.042</td>
<td>-0.053</td>
<td>0.802</td>
<td>-3.015</td>
<td>0.832</td>
</tr>
</tbody>
</table>

### Table 8: Correlation coefficients for canonical variables V and microbial variables

<table>
<thead>
<tr>
<th>Factor</th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
<th>V4</th>
<th>V5</th>
<th>V6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria content (BC)</td>
<td>-2.803</td>
<td>0.647</td>
<td>0.315</td>
<td>-4.934</td>
<td>-24.984</td>
<td>0.410</td>
</tr>
<tr>
<td>Fungi content (PC)</td>
<td>-67.958</td>
<td>-0.660</td>
<td>0.636</td>
<td>-24.382</td>
<td>-63.781</td>
<td>31.077</td>
</tr>
<tr>
<td>Actinomycetes content (AC)</td>
<td>124.470</td>
<td>-1.327</td>
<td>-0.056</td>
<td>43.735</td>
<td>117.941</td>
<td>-40.503</td>
</tr>
<tr>
<td>Total microbe content (TM)</td>
<td>1.744</td>
<td>-0.308</td>
<td>0.080</td>
<td>-4.792</td>
<td>10.252</td>
<td>2.231</td>
</tr>
</tbody>
</table>
microbial community. Nevertheless, because the wetland degradation significantly changes the soil water content, the physical and chemical properties of the soil are modified, leading to variation of soil microbial content. In our previous studies, we have found that the soil microbial carbon metabolism and community structure of meadow and marsh meadow Deyeuxia angustifolia wetlands were strongly increased compared to marsh wetland. These results show that after wetland has degraded, the change of soil water content results in the change of physical and chemical properties of soil, ultimately leading to the change of soil microbial function and structure.

Influence of Wetland Degradation on Soil Microbial Diversity

Various diversity indices can be calculated to estimate species richness and evenness. The diversity index value reflects the diversity of a microbial community (Smolander and Veikko, 2002; Wu et al., 2013). This study showed that under various degradation stages, the community structure of soil microbes has undergone significant changes. The Shannon index of meadow Deyeuxia angustifolia wetland was higher than that of marsh wetland which again was higher than that of marsh meadow wetland (Table 4). Thus, wetland degradation has led to changes of soil microbial community structure. In a previous analysis of carbon metabolism and microbial function diversity for these three types of soil, it was shown that the Shannon indices were highest for meadow wetland, lower for marsh meadow and lowest for marsh Deyeuxia angustifolia wetland, which conflicts with our results. That study was based on results from carbon metabolism and microbial function only, which has certain limitations. On the other hand, although PLFA analysis as applied here detects microorganism more broadly, possibly providing more accurate results, our study is limited because not all detected PLFA profiles could be attributed to specific microorganisms. From the results of this research, one could conclude that degradation of Deyeuxia angustifolia wetland results in an increase in soil microbial amounts and diversity, which could be regarded favorable changes. Nevertheless, it is worth noticing that wetland has a very important ecological role as a carbon sink (Paul and Beaucham, 1996). As the water level of wetland drops, the surface is better aerated, so that aerobic soil microbes are favored (Paul and Beaucham, 1996). The biogeochemical cycle of wetland is changed, leading to increased emission of greenhouse gases and modification of wetland ecosystem functions. These facts have become a research focus. In addition, once degraded, the wetland ecosystem is modified, which has a long-term effect on the composition of wetland plants and may significantly alter wetland ecosystem functions. Therefore, the reduction of wetland water levels is overall unfavorable. Actions must be taken to conserve wetlands in order to fully utilize their ecological function.

Conclusion

This study report that wetland degradation results in an increase of the soil nutrient content, and significantly increases soil microbial biomass. The latter was assessed by PLFA analysis. After wetland degradation, the pH, water content, and total nitrogen content of the soil are the main environmental factors affecting soil microbial biomass. The biomass of Actinomycetes and fungi is particularly affected, while total bacteria is least affected. These results suggest that the change of soil environment can significantly influence the soil microbial biomass and community composition. However, the results depend on the detection method employed. Therefore, a diverse variety of methods needs to be employed, of which PLFA is a valuable addition for future research.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (NO.31470019, 31500410); the National Key R&D Program of China (2016YFC0500405-02); Fund of Heilongjiang academy of sciences (STJB1601; STJB1603).

References


(Received 22 January 2018; Accepted 22 March 2018)