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Full Length Article

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

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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 (PFLA). 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 PFLA 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 protozoavaried between the soil types with lower values in marsh wetlandthan the other soil types. Canonical correlation analysis identified thatsoil 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 diverseas 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 coveragefrom 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 aremainly 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 byphysical 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 analysisof PFLA provides a good measure of living microbial populations (Lovett *et al*., 1982). By analyzing the type and composition ratio of PFLA< the precise microbial community structure and diversity can objectively and quantitatively be described. Here we analyzed soil microbial

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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 theresearch stations in Honghe National Nature Reserve, the institute of Natural and Ecology, Heilongjiang Academy of Sciences, China. The research stations are located at analtitude of 50 to 65 m, where the annual mean temperatureis 1.9℃, and the effective accumulated temperature (≥10℃) is 2165 ~ 2624℃. 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 arandom 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℃ for analysis of the diversity of the soil microbial community. The other part was airdried, 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 PFLA.

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 m*M* 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℃ 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 acidswas determined by spectrum analysis using an Agilent 6850Nin combination withSherlock MIS 4.5 software. As internal marker n-hexane (C19:0) was used, the mixture was measured by gas chromatography (Agilent 6890). The concentration of each fatty acid was determined based on the concentration of carbon internal marker 19:0, and expressed as nmol/g dry soil.

Physical and Chemical Property of Soil

The water content in the soil was measured by comparing fresh soil weight with the weight after drying. The pH was measured after mixing soil with deionized water in a water-to-soil ratio of 2.5:1. The organic carbon content of the soil was measured by a carbon nitrogen analyzer (Jena-2100S, Germany). The total nitrogen content was measured by the semi-micro Kjeldahl method and the nitrate nitrogen content was obtained by the phenol-two-sulfonic acid method. Lastly, the nitrogen content in the form of ammonium was measured by potassium chloride extraction/indigo blue colorimetry.

Data Analysis

The PLFA constituents were calculated by the method of Qian *et al.* (2008). The fatty acid data were converted to concentration (nmol/g dry soil) using the equation:

$$C(x) = (A:B)xCxVxF$$

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 esterof 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 tomicrobe types, together with the detected concentrations in the three different soil types analyzed (Table 3). The carbon chain length varied from 11 ~ 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 bacteriainferred from PLFA data suggested a significantly (P<0.01) higher abundance in marsh and mash meadowwetland 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.

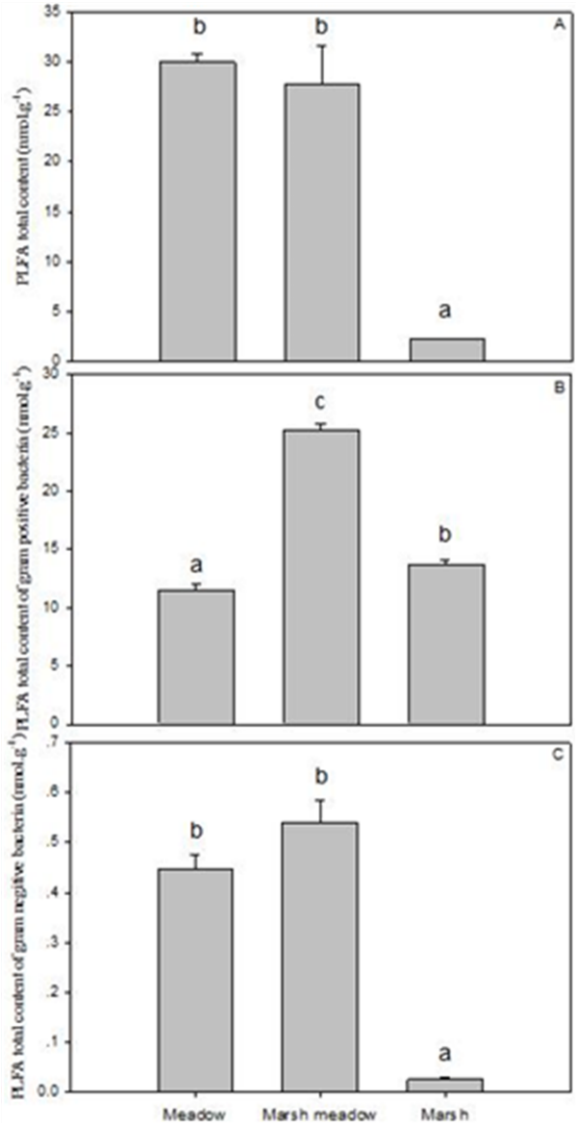

Fig. 1: Total PLFA Content of Soil Microorganisms in *Deyeuxia angustifolia* wetlands at different degradation stages. Panel A: total microbial content; PanelB: Gram-positive bacteria; Panel C: Gram-negative bacteria. Significance is shown as * for P<0.05, ** for P<0.01

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

Wetland Type	Location	Main plant types
Meadow *Deyeuxia angustifolia* wetland (w0)	47°47'21"N, 133°37'51"E	*D. angustifolia, Stellaria radian, Anemone dichotoma, Lathyrus quinquenervius, Thalictrum simplex*
Marsh meadow wetland (w1)	47°45'39"N, 133°37'04"E	*D. angustifolia, Carexappendiculata, Lathyrus quinquenervius, Carex. miyabeivar. Maopengensis*
Marsh wetland (w2)	47°47'16"N, 133°37'43"E	*D. angustifolia, Carex pseudo-conicam, C. miyabeivarmaopengensis, Glyceria spiculosa, Calamagrostis neglecta, Salix rosmarinifolia, Salix myrtilloides*

Table 2: PLFA characteristics of microbial groups

Microbial group	Phospholipids fatty acid biomarkers*
Bacteria	Saturated or mono unsaturated fatty acid with an ester chain linked to glycerol, e.g., 15:0, i15:0, a15:0, 16:0, i16:0, 16:1ω5, 16:1ω9, 16:1ω7t, 17:0, i17:0, a17:0, cy17:0, 18:1ω5, 18:1ω7, 18:1ω7t, i19:0, a 19:0 and cy19:0
Aerobes	i15:0, a15:0, 15:0, i16:0, 16:1ω9, 16:1ω7t, i17:0, a17:0, 17:0
Anaerobes	cy17:0, cy19:0
Gram-positive bacteria	Multiple branched fatty acids(iso－, anteiso－) e.g., 18:1 w7c 11 - methyl, 16:0 iso, 15:0 iso, 15:0 anteiso, 17:0,11:0 3 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
Gram-negative bacteria	Variety of hydroxy fatty acids (mono fatty acids, cyclic fatty acids etc.) e.g.16:1 w5c, 14:1 w5c, 16:00, 18:00, 13:0 iso, 17:1 w8c
Methane-oxidizing bacteria	16:1 w5c
Fungi	Contain unique phospholipid fatty acids, e.g., 18:1ω9,18:2ω6,18:3ω6,18:3ω3
Protozoa	20:3ω6,20:4ω6
Bacterial taxonomic group	
Actinomycetes spp.	10Me16:0, 10Me17:0, 10Me18:0, 17:0 10 – methyl, 18:0 10 – methyl, TBSA
Pseudomonas spp.	14:1 w5c, 16:00,
Arthrobacter spp.	17:00
Cellulomonas spp.	18:1 w7c 11 – methyl
Flavobacterium spp.	13:0 iso

*i: iso-, a: anteiso, cy: cyclopropyl and Me: methyl branching fatty acids. ω: aliphatic end, c: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

PLFA name	Microbial types	Meadow wetland (nmol/g)	Marsh meadow wetland (nmol/g)	Pristine wetland (nmol/g)
12:00	Bacteria	0.0142	0.0386	0
14:00	Bacteria	0.4880	0.5182	0.0296
15:1w6c	Bacteria	0.1855	0.1806	0.0251
15:00	Bacteria	0.4505	0.4907	0.0228
19:0 iso	Bacteria	0.0516	0.0539	0
20:00	Bacteria	0.3226	0.3240	0.2706
16:0 iso	Gram-positives	1.5322	1.7973	0.0517
15:0 iso	Gram-positives	3.7914	3.5318	0.1356
15:0 anteiso	Gram-positives	3.7424	3.3489	0.1530
11:0 3 OH	Gram-positives	0	0.0263	0
18:0 iso	Gram-positives	0.1984	0.2149	0.0309
17:0 anteiso	Gram-positives	1.1829	1.1503	0.0968
16:0 anteiso	Gram-positives	0.1426	0.1630	0.0366
17:1 w8c	Gram-negatives	0.4417	0.5534	0.0282
17:0 iso	Aerobes/Gram-positives	1.0836	1.0333	0.0633
14:0 iso	Aerobes	0.2674	0.2890	0
16:1 w5c	Methane-oxidizing Gram-negatives	1.2031	0.9964	0.0528
13:0 iso	*Flavobacterium spp.*	0.0316	0.0567	0
14:1 w5c	*Pseudomonas spp.*	0.0402	0.0442	0
16:00	*Pseudomonas spp.*	5.7186	5.4822	0.4144
17:00	*Arthrobacter spp.*	0.4472	0.4341	0.0276
17:0 10 – methyl	*Actinomycetes*	0.5808	0.7377	0.0160
18:00	*Hydrogenobacter*	1.3893	1.4376	0.1122
18:1 w7c 11 - methyl	*Cellulomonas spp.*	0.5007	0.4890	0.0483
18:0 10 – methyl, TBSA	*Actinomycetes*	1.3887	1.5168	0.0483
18:3 w6c (6,9,12)	Fungi	0.2284	0.1571	0.0258
18:1 w9c	Fungi	4.4988	6.0597	0.1128
20:4 w6,9,12,15c	Protozoa	0.2457	0	0.1647
20:2 w6,9c	Protozoa	0.1788	0.1564	0.3099

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

Wetland type	Pielou uniformity index*	Shannon-Weiner index*	Simpson index*
Meadow *Deyeuxia angustifolia* wetland (w0)	0.79 ± 0.17^{a}	2.63 ± 0.17^{a}	0.90 ± 0.01^{a}
Marsh meadow *Deyeuxia angustifolia* wetland (w1)	0.78 ± 0.18^{a}	2.60 ± 0.18^{a}	0.89 ± 0.00^{a}
Marsh *Deyeuxia angustifolia* wetland (w2)	0.87 ± 0.29^{b}	2.71 ± 0.21^{b}	0.91 ± 0.00^{a}

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

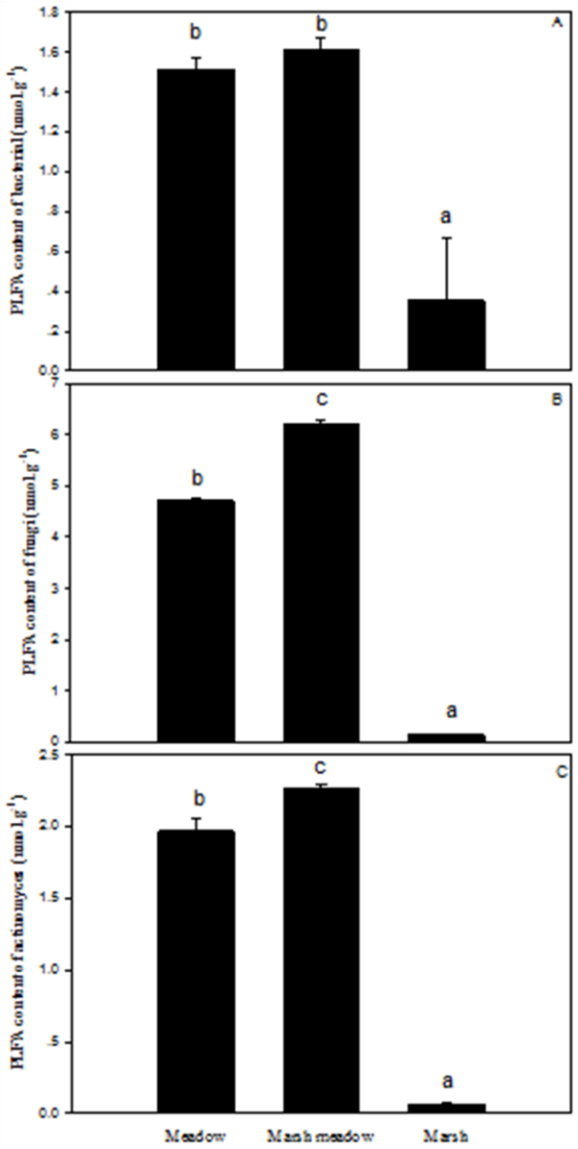

Fig. 2: Soil content of microorganisms as inferred by PLFA detection in *Deyeuxia angustifolia* wetlands. Panel A shows Bacteria, panel B fungi and panel C *Actinomycetes*. Significance is shown as a, b, c for $P<0.05$

The detected fatty acids suggest differences in presence of bacteria, fungi, *Actinomycetes*, and protozoa 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 diversitywhich 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.

Table 5: Canonical variable correlation coefficient and significance test

Group	Wilk's	Chi-Sq	DF	Sig	r
1	0	.000	36.000	.000	1
2	0	148.246	25.000	.000	0.98
3	0	95.221	16.000	.000	0.763
4	0	45.003	9.000	.000	0.458
5	.686	.565	4	.967	
6	.979	.032	1	.967	

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 content (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:

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

U_2=-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 $U_2$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:

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.

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

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

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

Factor*	U1	U2	U3	U4	U5	U6
pH	6.576	-0.234	0.836	0.949	0.784	2.437
Organic carbon (OC)	-0.234	-1.351	1.18	8.778	6.25	11.471
Total nitrogen (TN)	-0.836	0.705	2.314	3.828	2.314	3.828
Nitrate nitrogen (NN)	-0.949	-0.498	-0.600	-8.483	-1.335	-11.475
Ammonium nitrogen (AN)	0.784	0.474	0.830	13.615	-4.838	-0.814
Soil water content (WC)	-2.437	-0.042	-0.053	0.802	-3.015	0.832

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

Factor	V1	V2	V3	V4	V5	V6
Bacteria content (BC)	-28.130	0.647	0.315	-4.934	-24.984	0.410
Fungi content (FC)	-67.958	-0.660	0.656	-24.382	-63.781	31.077
Actinomycetes content (AC)	124.470	-1.327	-0.056	43.736	117.941	-40.503
Total microbe content (TM)	1.744	-0.398	0.308	-4.792	10.252	2.231

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 createsthe largest impact on *Actinomycetes* in the soil microbial community, followed by effects on fungi and bacteria (Table 8).

Discussion

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 soil increases, whichsupports 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 succession stages, 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 soilat different degradation stages reached a maximumat 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 influenced 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 changedsoil microbial community. The soil investigated in this work ismainly covered by *D. angustifolia*. Thus, differences in plant type are not the responsible for the observed differences in soil 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 tomarsh 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.

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 of marsh wetland which again was higher than 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 meadowand 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 detectsmicroorganismson a broader scale, possibly giving 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 resulted 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 of 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.

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References

Andersen, R., L. Grasset and M.N. Thormann, 2010. Changes in microbial community structure and function following Sphagnum peatland restoration. *Soil Biol. Biochem.*, 42: 291–301

Anderson, S.A., P.T. Northcote and M.J. Page, 2010. Spatial and temporal variability of the bacterial community in different chemotypes of the New Zealand marine sponge *Mycale hentscheli*. *FEMS Microbiol. Ecol. Bann.*, 72: 328–342

Cai, X.B. and Y.Q. Zhang, 2008. Characteristics of soil fertility in alpine steppes at different degradation grades. *Acta Ecol. Sin.*, 28:1034–1044

Dentener, F., J. Drevet, J.F. Lamarque, I. Bey, B. Eickhout, A.M. Fiore, D. Hauglustaine, L.W. Horowitz, M. Krol, U.C. Kulshrestha, C. Galy-Lacaux, S. Rast, D. Shindell, D. Stevenson, T. Van Noije, C. Atherton, N. Bell, D. Bergman, T. Butler, J. Cofala, B. Collins, R. Doherty, K. Ellingsen, J. Galloway, M. Gauss, V. Montanaro, J.F. Müller, G. Pitari, J. Rodriguez, M. Sanderson, F. Solmon, S. Strahan, M. Schultz, K. Sudo, S. Szopa and O. Wild, 2006. Nitrogen and sulfur deposition on regional and global scales: A multimodel evaluation. *Glob. Biogeochem. Cycl.*, 20: 16615–16636

Drenovsky, R.E., G.N. Elliott and K.J. Graham, 2004. Comparison of phospholipid fatty acid (PLFA) and total soil fatty acid methyl esters (TSFAME) for characterizing soil microbial communities. *Soil Biol. Biochem.*, 36: 1793–1800

Galloway, J.N., F.J. Dentener and D.G. Capone, 2004. Nitrogen cycles: past, present, and future.fruit present and future. *Royal Hortic. Soc.*, 153–226

Gong, C., C. Song and W. Tan, 2015. Effects of marshland reclamation on soil microbial properties in the sanjiang plain, northeast China. *Ecol. Environ. Sci.*, 24: 972–977

Kazda, M., 1990. Indications of unbalanced nitrogen nutrition of Norway spruce stands. *Plant Soil*, 128: 97–101

Lei, H., C.T. Wang and G.X. Wang, 2014. Changes in the activities of soil enzymes and microbial community structure at different degradation successional stages of alpine meadows in the headwater region of Three Rivers, China. *Acta Pratac. Sin.*, 23: 8–19

Liu, F., S. Ye and Y. Tang, 2007. Analysis of microbial community structure in coastal wetland soil of the Yellow River Delta. *Chin. J. Appl. Environ. Biol.*, 13: 691–696

Lovett, G.M., W.A. Reiners and R.K. Olson, 1982. Cloud droplet deposition in subalpine balsam fir forests: hydrological and chemical inputs. *Science*, 218: 1303–1304

Lu, Q., K.M. Ma and J.Y. Zhang, 2007. Soil nutrients of degraded wetland and farmland in Sanjiang plain. *J. Ecol. Rural Environ.*, 23: 23–28

Paul, J.W. and E.G. Beauchamp, 1996. Soil microbial biomass C, N mineralization, and N uptake by corn in dairy cattle slurry-and urea-amended soils. *Can. J. Soil Sci.*, 76: 469–472

Qian, W., L. Qimei and Z. Xiaorong, 2008. Application of PLFA analysis in determination of soil microbial community structure in woodland, cropland and grassland in farmland-pasture interleaving zone of north china. *Acta Pedol. Sin.*, 45: 321–327

Smolander, A. and K. Veikko, 2002. Soil microbial activities and characteristics of dissolved organic C and N in relation to tree species. *Soil Biol. Biochem.*, 34: 651–660

Sui, X., R.T. Zhang and L.B. Yang, 2017. Differences in the microbial population associated with three wetland types in the Sanjiang plain, Northeast China. *Appl. Ecol. Environ. Res.*, 15: 79–92

Sui, X., R.T. Zhang and H.X. Zhong, 2015. Study on bacterial diversity of *Deyeuxia angustifolia* wetland by application of high-throughput sequencing technology in Sanjiang plain. *Soils*, 47: 919–925

Wieten, A.C., A.D. Parker and D.G. Uzarski, 2012. Great Lakes coastal wetland habitat use by seven turtle species: influences of wetland type, vegetation, and abiotic conditions. *Wetl. Ecol. Manage.*, 20: 47–58

Wright, R.F., J.G.M. Roelofs and M. Bredemeier, 1995. Nitrex-response of coniferous forest ecosystems to experimentally changed deposition of nitrogen. *For. Ecol. Manage.*, 71: 163–169

Wu, Z.Y., W.X. Lin and Z.F. Chen, 2013. Characteristics of soil microbial community under different vegetation types in Wuyishan national nature reserve, East China. *Chin. J. Appl. Ecol.*, 24: 2301–2309

Zhao, X.L. and G.S. Zhou, 2006. The characteristics of soil microbe in Panjin reed wetland. *J. Meteorol. Environ.*, 22: 64–67

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