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Changes in the soil microbial phospholipid fatty acid profile with depth in three soil types of paddy fields in China

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ABSTRACT

We know very little about microbial communities in deeper soil horizons, especially the microbial characteristics along soil depth profiles in paddy fields. In this study, we used a phospholipid fatty acid (PLFA) analysis to investigate the vertical distribution of the microbial community, and to identify the environmental factors that contributed to changes of the microbial community along a soil depth gradient. Samples were collected from the soil surface to 1 m in depth from three soil types of paddy fields in three regions. The results showed that total PLFAs and Shannon's diversity index decreased significantly with increasing soil depth, and they exhibited relatively low values at soil depths less than 40 cm. In the paddy soil profile, the relative microbial abundance was in the order bacteria > actinomycetes > fungi. Generally, the relative abundances of bacteria and fungi significantly decreased at 0–40 cm depths and then increased at 40–100 cm depths; however, the relative abundance of actinomycetes usually increased at 0–40 cm depths, and then decreased significantly with increasing soil depth. The proportion of Gram-positive bacteria was higher than that of Gram-negative bacteria in deeper soils. Canonical correspondence analysis and Pearson's correlation analysis indicated that nutrient resources and soil water content were more closely correlated with the changes of the microbial community structures along the soil depth gradient than soil pH, elevation, and climate, and were the primary drivers of the microbial community composition along the soil depth gradient.

1. Introduction

Microorganisms play an important role in agricultural ecosystems, mainly in terms of sustainability and the quality of agricultural soils (Kennedy and Smith, 1995). However, most soil microbiology studies have focused on the 0–20 cm soil depth; thus, we know very little about the changes of microbial communities with soil depth in agricultural soils (Bao et al., 2015; Yu et al., 2015). As we know, the nutrient availability of surface soils is usually greater than that of subsurface soil because of the input of crop root exudates, surface litter, and root detritus in agricultural systems. Furthermore, fertilizer applications enlarge the nutrient gap between surface and subsurface soils (Li et al., 2014). Nutrient availability, pH, soil texture, temperature, and moisture content can vary considerably with soil depth (Brady and Weil, 2002). Differences in physical and chemical parameters along a soil depth gradient allow for the proliferation of diverse microbial communities. Deeper soil layers may contain microbial communities that have adapted to this environment, and these communities may be distinct from surface communities (Bai et al., 2015).

Many environmental factors that influence microbial communities have been reported, including pH (Griffiths et al., 2011), soil texture (Chodak and Niklińska, 2010), nutrient availability (Bowles et al., 2014), water content (Moche et al., 2015; Praeg et al., 2014), and temperature (Wang et al., 2014). These environmental parameters usually exhibit spatial heterogeneity; thus, the distributions of the microbial community along a soil depth gradient will vary greatly with spatial location. Some studies have demonstrated that microbial biomass (Sun et al., 2016) and diversity (Huang et al., 2013) decrease with increasing soil depth, and that the microbial community structure differs between surface and subsurface soils (Fierer et al., 2003). These results mostly came from small-scale or single-soil type studies, which cannot be extrapolated to larger scales when attempting to understand microbial community characteristics along a soil depth gradient.

Phospholipid fatty acids (PLFAs) are good indicators of living organisms, and they can be used to adequately detect rapid changes in soil microbial communities because they are essential components of the membranes of all living microbes, and they rapidly degrade as cells die (White et al., 1979). Furthermore, different PLFAs can be used as

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biomarkers for major microbial groups, e.g., fungi, bacteria, and actinomycetes (Hao et al., 2008). PLFA analyses have been widely used to evaluate microbial community composition and biomass (Mahmoudi et al., 2015; Zhang et al., 2015).

In this study, we mainly used the PLFA method to investigate how the microbial community structure varies along a depth gradient at three different regions/soil types in China, and to explain the factors controlling this variation.

2. Materials and methods

2.1. Site description and soil sample collection

Soil samples were collected from three regions, Hailun, Changshu, and Yingtan, which are located in the north, middle, and south of China, respectively. The distance between Hailun and Changshu is approximately 2500 km, and Changshu and Yingtan are 8000 km apart. Soil type, cropping pattern, annual precipitation, and number of frost-free days per year at these three regions are as follows: Hapli-Udic lsohumosols, one crop per year, 530 mm annual precipitation, and 149 frost-free days for Hailun; Gleyic-Stagnic Anthrosols, two crops (wheat-rice) per year, 1028 mm annual precipitation, and 235 frost-free days for Changshu; and Argi-Udic Ferrosols, two crops (rice-rice) per year, 1795 mm annual precipitation, and 264 frost-free days for Yingtan. Soil type is classified according to the Chinese Soil Taxonomy (Cooperative Research Group on Chinese Soil Taxonomy (CRGCST), 2001).

The samples were collected after the late rice harvest (October–November 2014). In each region, five replicates were collected from five randomly selected sites (at least 3 km apart from each other). These sites have less variation in site characteristics, i.e., soil type, cropping system, and climatic conditions. In each site, soil samples were taken vertically using an auger at the following depth intervals (cm): 0–10, 10–20, 20–40, 40–60, 60–80, and 80–100 at six randomly sampling points (20 m apart), and six subsamples from each depth were mixed and pooled as one composite sample. Thus, there were 90 samples, i.e., 30 samples from Hailun (126°51′–126°58′ E, 47°31′–47°33′ N, 168–226 m elevation), 30 samples from Changshu (120°35′–120°40′ E, 31°30′–36°33′ N, 2–6 m elevation), and 30 samples from Yingtan (116°54′–116°56′ E, 28°10′–28°13′ N, 34–62 m elevation).

2.2. Laboratory analyses

Soil water content was determined using the gravimetric method after drying the soil to a constant weight at 105 °C. Dissolved organic carbon (DOC) was determined by a Multi N/C 3100 analyzer (Analytik Jena AG, Jena, Germany). Concentrations of NH_4^+-N and NO_3^--N in filtered 2 M KCl extracts from fresh soil were measured by AA3 Continuous Flow Analyzer (Seal Analytical, Germany). Soil total C and N concentrations were measured with a vario MICRO cube (Elementar Corporation, Germany).

The soil microbial community was characterized using a PLFA analysis. PLFAs were extracted from the soil using the procedure of Petersen and Klug (1994), with minor modifications. Briefly, total lipids were extracted from 8 g of freeze dried soil sample using a 30.4-mL mixture of methanol/chloroform/citric acid buffer (0.15 M, pH 4) (2:1:0.8, v/v/v). Polar lipids were separated from neutral and glycolipids on a silica column (Cleanert Silica cartridge, 500 mg 6 mL^{-1}, Agela Technologies Inc.). After mild alkaline methanolysis, polar lipids were converted to fatty acid methyl esters. The individual fatty acid methyl esters in the sample were analyzed by an Agilent 7890A gas chromatograph with MIDI peak identification software. The fatty acid 19:0 was added as an internal standard. Based on previously published PLFA biomarker data, the following PLFA biomarkers were considered to represent Gram-positive (G^+) bacteria: i14:0, i15:0, a15:0, i16:0, a16:0, i17:0, and a17:0. Gram-negative (G^-) bacteria were represented 16:1ω9c, cy17:0, 18:1ω5c, 18:1ω7c, and cy19:0 (Frostegård and Bååth, 1996), and total bacteria were calculated as the sum of the G^+ and G^- bacteria. The fatty acids 18:2ω6 and 18:1ω9c were used as markers for fungal PLFAs (Bååth and Anderson, 2003), and 10Me16:0, 10Me17:0, and 10Me18:0 were used as markers for actinomycete PLFAs (Zelles, 1997). Total PLFAs were calculated as the sum of all PLFAs.

2.3. Statistical analysis

Shannon's index (H) was calculated to examine the diversity of the microbial community (Shannon and Weaver, 1963). Statistical analyses to assess the effects of region and soil depth on the measured soil physico-chemical parameters and soil microbial PLFAs were conducted by two-way analysis of variance (ANOVA) using Tukey's honestly significant difference (HSD) multiple comparison test ($P < 0.05$) with SPSS for Windows version 11.5 software (SPSS Inc., Chicago, IL, USA). Pearson's correlation analysis between total PLFAs, the relative abundances of microbial groups, and environmental factors were conducted with SPSS 11.5 software. A canonical correspondence analysis (CCA) was conducted by Canoco for Windows (Version 4.5) with the Monte Carlo permutations test (499 permutations) to determine whether the microbial community structure could be correlated to environmental factors.

3. Results

3.1. Soil properties

The data shown are expressed as the means ± S.D. of the five replicates. Two-way ANOVA showed that soil depth, region, and their interaction had significant effects on the soil properties, except for NH_4^+-N (Table 1). The total C and total N contents, the NO_3^--N and DOC concentration, pH, and the soil water content differed significantly among six depths (Table 1). The total C contents ranged from 1.66 to 34.90 g kg^{-1}, and the total N contents ranged from 0.32 to 2.70 g kg^{-1} (Table 2). The soil total C and N contents significantly decreased at 0–60 cm depths (Table 2). The DOC concentration and pH significantly increased in the 0–20 cm and 0–40 cm depths, respectively. The soil water content and the NO_3^--N concentration significantly decreased at 0–20 cm depths. However, the NH_4^+-N concentration and the C/N ratio did not change significantly with soil depth (Table 1). The C/N ratio, the total C and total N contents, the DOC concentration, and the soil water content differed significantly among

Table 1
Two-way ANOVA analysis of soil properties and microbial groups at six soil depths in three regions, each with five replicates (n = 90). The data in bold indicate soil properties and microbial groups that were not affected by soil depth, region, or their interaction ($P > 0.05$).

	Depth		Region		Depth × region	
	F	*P*	*F*	*P*	*F*	*P*
pH	21.60	<0.0001	215.17	<0.0001	5.40	<0.0001
Total C	91.69	<0.0001	252.41	<0.0001	6.55	<0.0001
Total N	78.91	<0.0001	86.21	<0.0001	3.59	0.001
Soil water content	8.79	<0.0001	11.15	<0.0001	**0.63**	**0.780**
DOC	7.05	<0.0001	11.86	<0.0001	**0.71**	**0.707**
NO_3^--N	4.57	0.001	**2.21**	**0.069**	**1.80**	**0.076**
NH_4^+-N	**0.917**	**0.475**	**0.942**	**0.395**	**0.68**	**0.735**
C/N	**2.14**	**0.070**	143.02	<0.0001	4.56	<0.0001
Total PLFAs	70.38	<0.0001	11.83	<0.0001	5.30	<0.0001
Bacteria	10.77	<0.0001	17.39	<0.0001	2.65	0.008
Fungi	4.70	0.001	**1.22**	**0.301**	**1.38**	**0.204**
Actinomycetes	16.56	<0.0001	18.70	<0.0001	3.74	<0.0001
G^- bacteria	33.52	<0.0001	5.34	0.007	**0.972**	**0.475**
G^+ bacteria	31.00	<0.0001	13.90	<0.0001	**1.53**	**0.144**

DOC, dissolved organic carbon; G^- bacteria, Gram-negative bacteria; G^+ bacteria, Gram-positive bacteria; C/N, total C: total N ratio; PFLAs, phospholipid fatty acids