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Changes in soil microbial community structure and functional diversity in the rhizosphere surrounding mulberry subjected to long-term fertilization

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ABSTRACT

A long-term field experiment was used to evaluate the effects of different fertilizers (OIO, organic inorganic compound fertilizer specially formulated for mulberry; N, N fertilizer only; NPK, N, P and K fertilizer; NF, the control without fertilization) on microbial abundance, community structure and microbial functional diversity. The OIO treatment exhibited the highest bacterial and actinomycete levels, community diversity, Shannon indices and average well color development (AWCD). However, the N treatment resulted in the highest fungi levels. *Bacillus* sp. were the dominant bacteria in the OIO treatment. The carbohydrate and carboxylic acid utilization by microbes was highest in the OIO treatment, whereas the amino acid utilization was highest in the N treatment. Most microbial parameters were primarily correlated with the soil organic matter content. The principal component analysis of the DGGE profiles suggested that the culture-independent bacterial community was not really affected by the fertilization treatments but was significantly affected by the sampling time. In contrast, the carbon substrate utilization was significantly affected by the fertilization treatments but not by the sampling time. Our findings suggested that the soil microbial activities were significantly changed by long-term fertilization and that more attention should be devoted to seasonal shifts in the microbial community.

1. Introduction

Soil microorganisms play critical roles in nutrient cycling and soil structure maintenance (Doran and Zeiss, 2000; Lin et al., 2004), and their diversity is an important soil microbial parameter. It has been demonstrated that the soil microbial diversity is affected by different fertilizer management programs (Fox and MacDonald, 2003; Lupwayi et al., 2012; Sradnick et al., 2013). The influence of fertilizer applications on the soil microbial diversity is very complex and may be related to the fertilizer type, application pattern (application rates and durations), soil type and other factors (Yang et al., 2011; Lopes et al., 2011; Lupwayi et al., 2012; Chan et al., 2013). Inorganic fertilizers, particularly nitrogen (N), improve crop yields, but their application over long periods of time is believed to significantly influence the quality and productive capacity of soil (Acton and Gregorich, 1995; Biederbeck et al., 1996; Banerjee et al., 1999; Dennis et al., 2010). However, the available information is conflicting, and uncertainties remain regarding the long-term effects of inorganic fertilizers on the soil microbial community and functional diversity. Previous studies have shown that inorganic fertilizers increase biomass C and N (Lynch and Panting, 1982; Kanazawa et al., 1988; Goyal et al., 1992), but other studies have reported that long-term N application reduces the microbial functional diversity (Lovell et al., 1995; Sarathchandra et al., 2001). Another set of studies have suggested that a certain amount of N had no effect on the soil microbial biomass, community structure or functional diversity (Ogilvie et al., 2008; Zhang et al., 2008; Lupwayi et al., 2010, 2011, 2012). The application of inorganic fertilizers has been shown to have a direct effect on the composition of the soil microbial community and functional diversity (Katayama et al., 1998; Ruppel and Makswitat, 1998; Enwall et al., 2005; Chu et al., 2007; Ogilvie et al., 2008). However, the effects of inorganic fertilizer on soil properties, particularly the soil microbial abundance, community structure and functional diversity, are not well understood in mulberry plantations.

Mulberry (*Morus* spp.) leaves provide the sole source of food for silkworms (*Bombyx mori*). One of the main silk-producing regions

Abbreviations: AWCD, average well color development; N, N fertilizer; NF, the control without fertilization; NPK, N, P and K fertilizer; OIO, organic–inorganic compound fertilizer especially formulated for mulberry.

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in China is Hubei Province, but the mulberry-leaf yield is 40–50% lower than in that obtained in other leading silk-producing provinces, such as Guangxi, Guangdong and Zhejiang. Mulberry is usually planted on less-productive land in hilly and mountainous areas. A previous survey suggested that fertilizer application has become an important factor limiting leaf yield and quality in Hubei province (Chen et al., 2009). Farmers need to feed silkworms 3–4 times a year, which means that large leaf harvests are needed. To obtain large quantities of leaves, farmers often use only inorganic fertilizers, particularly N fertilizers, and do not use organic fertilizers. At present, nitrogen fertilizers are applied to mulberry plantations at the high rate of 454 kg ha^{-1} $year^{-1}$ in Hubei Province (Chen et al., 2009). However, few studies have investigated the effects of this high input level on the soil quality and on changes in the soil microbial properties (Yu et al., 2011). We performed a long-term field experiment with four fertilizer treatments to accurately investigate the influences of different fertilizers on the soil microbial characteristics. The soil microbial abundances, community structure, and microbial functional diversity were measured. Our objectives were to understand the change in microbial characteristics during different growth periods when different fertilizers were applied over a longer period of time and to provide a theoretical basis for fertilization management in mulberry plantations.

2. Materials and methods

2.1. Natural profile of the study site

The site of the long-term fertilization experiment was established in 1996 at the experimental farm of the Industrial Crops Institute, Hubei Academy of Agricultural Sciences, Hubei Province, China (30°35′N, 114°37′E, 50 m a.s.l.). The Yu71-1 mulberry (*Morus alba* L.) was chosen for the field study and planted at row × line spacings of 1.0 m × 1.5 m. This region has a typical subtropical monsoon climate with an average annual precipitation of 1269 mm and an average temperature between 15.8 °C and 17.5 °C. The soils are clay loam. Some of the initial characteristics of the surface (0–20 cm) soil were as follows: pH, 6.79 ± 0.08; soil organic matter (SOM), 1.82 ± 0.07%; available nitrogen, 42.9 ± 1.06 mg kg^{-1}; available phosphorus, 49.1 ± 2.11 mg kg^{-1}; and available potassium, 160.5 ± 3.15 mg kg^{-1}.

2.2. Experimental design

The experiment involved a completely randomized block design with four replicates. The treatments were OIO (application of organic–inorganic compound fertilizer especially formulated for mulberry), N (application of N fertilizer), NPK (application of inorganic N, P and K fertilizers) and NF (the control without fertilizer applications). The organic parts in the organic–inorganic compound fertilizer were chicken manure and humic acids, which were mixed and fermented at high temperature of 25–70 °C. Inorganic N, P and K fertilizers in the organic–inorganic compound fertilizer and inorganic fertilizer were applied as urea, superphosphate and potassium sulfate, respectively. The annual application rate of the organic–inorganic compound fertilizer was 3000 kg ha^{-1}. The nutrient ratio of the organic–inorganic compound fertilizer was 15:4:6 for N:P_2O_5:K_2O, and the organic matter content was 20%. The annual application rates for the NPK treatment were 450 kg N ha^{-1}, 120 kg P ha^{-1} and 180 kg K ha^{-1}, and the N application rate in the N treatment was 450 kg N ha^{-1}. The area of each plot was 66.7 m^2. The fertilizers were applied twice a year: 40% in the spring and 60% after pruning. The physicochemical properties of the rhizosphere soil in November 2011 and 2012 are shown in Table 1. The soil pH of soil was measured by preparing a slurry of 1:2.5 fresh soil to water (v/v) and using a pH meter (OHAUS, Starter 3C). The soil organic matter was determined by the standard Walkley–Black potassium dichromate oxidation method (Nelson and Sommers, 1982). The available N was measured by the alkali-hydrolysis and diffusion method. The available P was extracted with 0.5 M $NaHCO_3$ by the Olsen method (Blakemore et al., 1972). The available K was extracted with 1 M NH_4OAc (1:10 soil: solution ratio) for 1 h and analyzed by atomic absorption spectrophotometry (Lanyon and Heald, 1982).

8 to 15 soil samples per plot (using GPS to fix the position) were periodically collected at random from the mulberry rhizosphere and combined. Loose soil was removed from the roots, and the remaining soil that was strongly adhered to the roots was recovered as rhizosphere soil. The samplings took place at the end of May July, September and November 2011 and 2012. Part of the soil was stored at 4 °C for microbial abundance determination, another part of the soil was stored at −80 °C for soil microbiological analysis, and the remaining was air-dried, ground and passed through 1-mm and 2-mm mesh sieves for chemical analysis. The average environmental temperature and rainfall 10 days before sampling are shown in Table 2. The 2011 and 2012 seasons were close to normal in terms of temperature, but July and September were very dry.

2.3. Abundance measurements of culturable microbes and bacterial identification

The total numbers of cultured bacteria, fungi and actinomycetes were determined as the number of colony forming units (CFUs) on agar plates using dilution plate methods (Lin, 2010). The media used to separately culture the bacteria, fungi and actinomycetes were beef extract peptone medium, Czapek's medium and Gause's

Table 1
The physicochemical properties of mulberry rhizospheric soil treated with organic–inorganic compound fertilizer (OIO), nitrogen fertilizer (N), nitrogen, phosphorus and potassium mixed fertilizer (NPK) and no fertilizer (NF) in November 2011 and 2012.[1]

		pH (H_2O)	SOM[2] (%)	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
2011	OIO	6.32 ± 0.15^{ab}	1.93 ± 0.05^{a}	53.02 ± 3.59^{a}	41.91 ± 0.46^{ab}	146.00 ± 3.50^{a}
	N	5.57 ± 0.12^{b}	1.65 ± 0.02^{c}	45.50 ± 1.09^{b}	35.37 ± 0.99^{b}	73.00 ± 2.50^{c}
	NPK	6.46 ± 0.13^{ab}	1.74 ± 0.03^{b}	51.87 ± 2.12^{a}	44.99 ± 1.02^{a}	124.50 ± 2.00^{b}
	NF	6.54 ± 0.12^{a}	1.08 ± 0.12^{d}	23.86 ± 2.23^{c}	12.82 ± 3.13^{c}	55.50 ± 7.50^{d}
2012	OIO	6.65 ± 0.11^{ab}	2.03 ± 0.02^{a}	54.60 ± 3.09^{a}	50.43 ± 2.93^{a}	156.50 ± 5.50^{a}
	N	5.52 ± 0.14^{b}	1.63 ± 0.03^{b}	44.80 ± 3.01^{c}	24.21 ± 3.26^{c}	70.00 ± 8.50^{c}
	NPK	6.76 ± 0.09^{a}	1.72 ± 0.02^{b}	47.95 ± 5.12^{b}	42.21 ± 1.08^{b}	126.00 ± 2.00^{b}
	NF	6.43 ± 0.09^{ab}	1.04 ± 0.04^{c}	17.93 ± 1.23^{d}	9.64 ± 1.02^{d}	52.00 ± 8.00^{d}

[1] The data are expressed as the means ± SD ($n = 3$). The superscript letters that differ within a column indicate significant differences between treatments ($P < 0.05$).
[2] Soil organic matter (SOM).