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Responses of extracellular enzyme activities and microbial community in both the rhizosphere and bulk soil to long-term fertilization practices in a fluvo-aquic soil

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ABSTRACT

The influence of inorganic or organic fertilization on soil microbial ecology has been emphasized recently, but less is known about rhizosphere effects on extracellular enzyme activities and microbial community structure. Eleven extracellular enzymes involved in C, N, P, and S cycling and microbial community structure in both the rhizosphere and bulk soil samples from a long-term (31-year) fertilizer experimental field at the wheat reproductive stage were investigated by microplate fluorometric assay and phospholipid fatty acid analysis (PLFA), respectively. The samples were taken from six treatments: control (CK, without fertilization), fertilizer N (N), fertilizer N and P (NP), fertilizer N, P and K (NPK), organic manure (M), and organic manure plus fertilizer N, P and K (MNPK). Responses to inorganic or organic fertilizers in the rhizosphere were significantly different from those in the bulk soil. Except for NO_3^--N, thus, nutrient concentrations were generally higher in the rhizosphere than in the bulk soil. M and MNPK treatments greatly increased organic C, total N, NH_4^+-N and total S. Inorganic fertilizers (N, NP, and NPK) generally maintained or reduced most enzyme activities in the rhizosphere, but markedly increased these enzyme activities in the bulk soil. However, organic treatments (M and MNPK) enhanced most enzyme activities in both the rhizosphere and bulk soil. Higher total PLFA and lower ratios of bacteria to fungi and of actinomycetes to fungi were observed in the rhizosphere compared with the bulk soil. In the bulk soil, the ratios of bacteria to fungi and of actinomycetes to fungi were highest in the N treatment and lowest in the M treatment. However, in the rhizosphere there were no statistically significant differences in the abundance of bacteria, fungi and actinomycetes between the inorganic and organic treatments. Organic fertilization increased total PLFA and Gram+ to Gram− bacteria ratio in both the rhizosphere and bulk soil. Our results indicated that changes in fertilization regime had a greater impact on the bulk soil microbial community than in the rhizosphere.

1. Introduction

The rhizosphere, the volume of soil adjacent to and affected by plant roots (Sørensen, 1997), plays an important role in plant growth and soil fertility (Rovira, 1969). As soil microbes are often limited by energy in soils, root exudates such as organic acids, sugars and amino acids may stimulate the growth of microbial populations and the activities of extracellular enzymes capable of influencing biogeochemical cycling of C, N, P and S (Fontaine and Barot, 2005; Rovira, 1969; Stevenson and Cole, 1999). Fertilization, which is widely used to enhance soil fertility and crop yield, significantly affects soil biochemical and biological properties. The influence of fertilization on soil microbial ecology has been emphasized recently (Marschner, 2003; Yevdokimov et al., 2008; Zhong et al., 2010). However, most investigations have been conducted at a bulk soil scale or in short-term experiments, and as a result, there is still little available information on rhizosphere effects on extracellular enzyme activities and microbial community structure in agricultural soils as influenced by long-term practices.

From a functional perspective, the activities of extracellular enzymes produced by both microbes and plant roots are the primary biological mechanism of organic matter decomposition and nutrient cycling (Wittmann et al., 2004). Organic matter addition often leads to a rapid increase in the activities of various enzymes and reactivation of biogeochemical cycles in bulk soil (Bastida et al., 2007; Madejon et al., 2001). Inorganic N, P and K fertilizers also impact on the activities of soil enzymes (Böhme et al., 2005; Goyal et al., 1999). Most hydrolytic enzyme activities were increased by addition of N fertilizer in a forest soil, but the phenol oxidase activity dropped 40% compared to control plots (Saiya-Cork et al., 2002). Weand et al. (2010) emphasized that the effect of added N on enzymatic activities in a soil changes depending on the nature of the dominant substrates (labile or recalcitrant). Compared to numerous studies on enzyme activity in bulk soil, less effort has been expended on determining how long-term fertilization affects rhizosphere

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enzyme changes. In general, soil enzyme activities are lower in bulk soil than in the rhizosphere, as a result of microbial activity induced by root exudates, or because of the release of enzymes from roots (Badalucco and Kuikman, 2001). However, Phillips and Fahey (2008) found that rhizosphere effects on microbial activities and nutrient availability could be reduced by fertilizer addition in nutrient-poor forest soil, which he considered to be a result of fertilizer-induced shifts in the belowground C supply.

Most studies have found obvious changes in soil microbial communities after addition of organic or inorganic fertilizer amendments (Enwall et al., 2005; Marschner, 2003; Peacock et al., 2001). It is generally recognized that organic manure addition tends to increase the total microbial biomass, though the responses of specific groups such as Gram-positive bacteria, Gram-negative bacteria and fungi vary. For instance, organic manure additions often result in increased or altered fungal populations (Bastida et al., 2007; Elfstrand et al., 2007), altered populations of arbuscular mycorrhizal fungi (Corkidi et al., 2002), shifts in Gram-positive and Gram-negative bacteria (Marschner, 2003; Peacock et al., 2001), and increased fungi/bacteria ratios (Elfstrand et al., 2007). Importantly, the response of the microbial community structure to organic manure additions tends to be based on differences in the carbon amount or quality of the organic amendments (Elfstrand et al., 2007). Changes in the soil microbial community structure are also observed after additions of inorganic N, P and K fertilizers (Phillips and Fahey, 2008; Yevdokimov et al., 2008; Zhang et al., 2007). Many studies have indicated that rhizosphere community structure and function are mainly influenced by soil and plant factors (Carelli et al., 2000; Marschner et al., 2004). However, the ecological consequences of the application of various fertilizers in the rhizosphere are unclear, because of the poor understanding of how changes in nutrient availability impact on plant and soil microbial processes (Hobbie et al., 2002; Phillips and Fahey, 2007). Fertilizer additions possibly result in decreased carbon allocation to roots and subsequent decreases in microbial respiration in the rhizosphere (Phillips and Fahey, 2007). In another study, Buyer et al. (2010) reported that a vetch cover crop increased the amount and proportion of Gram-negative bacteria, fungi, and arbuscular mycorrhizal fungi in the rhizosphere of tomato plants.

The present study was conducted to examine how enzyme activity and microbial community structure differs between the rhizosphere and bulk soil in a farmland ecosystem, and how each responds to long-term fertilization. Since Marx et al. (2001) and Saiya-Cork et al. (2002) used fluorometric MUB-linked substrates to measure soil enzymes, this method has become popular in soil studies, because it is very sensitive and allows a high-throughput analysis of enzymatic activities (Deforest, 2009; Wittmann et al., 2004). Phospholipid fatty acid (PLFA) profiles were used to estimate the microbial community structure. We hypothesized that the rhizosphere and bulk soil would have different microbial communities with distinct enzyme activities after long-term fertilizer treatments, and that fertilization would influence rhizosphere effects on microbial community structure and function.

2. Material and methods

2.1. Field design and sampling

The study was conducted in the North China Plain, which is a major grain producing area in China. The calcareous fluvo-aquic soil is a widespread soil type in the North China Plain. In order to illustrate the effect of long-term fertilization on soil quality and food production, a long-term field experiment (incorporating application of inorganic/organic fertilizers and a control treatment) was initiated in 1979 at Malan Farm, Hebei province, China (37°55′N,115°13′E). At the start of the experiment, the soil had a pH (H_2O) of 7.8, 1.1% organic matter, 1.80 g kg^{-1} total N, and 5.0 and 87.0 mg kg^{-1} of available P and K, respectively. The site has a temperate and monsoonal type climate with annual average temperature and precipitation being 12.6 °C and 490 mm, respectively.

The experiment had winter wheat and summer maize rotations with a completely randomized design with twelve treatments and three replicates (Xia et al., 2008). The plot size was 80 m^2. For this study, six treatments were selected as follows:

1) Soil without fertilizer (control, CK)
2) Inorganic fertilizer treatment (N) corresponding to 150 kg N (urea) ha^{-1}.
3) Inorganic fertilizer treatment (NP) corresponding to 150 kg N (urea) ha^{-1} and 150 kg P_2O_5 (superphosphate) ha^{-1}.
4) Inorganic fertilizer treatment (NPK) corresponding to 150 kg N (urea) ha^{-1}, 150 kg P_2O_5 (superphosphate) ha^{-1} and 150 kg K_2O (KCl) ha^{-1}.
5) Farmyard manure compost (M), 3.75×10^4 kg ha^{-1}, containing straw bedding impregnated with liquid and solid manure.
6) Farmyard manure compost and inorganic fertilizer treatments (MNPK) corresponding to 3.75×10^4 kg cattle manure compost ha^{-1}, 150 kg N (urea) ha^{-1}, 150 kg P_2O_5 (superphosphate) ha^{-1} and 150 kg K_2O (KCl) ha^{-1}.

The manure compost had 120 g kg^{-1} organic matter; 5.0 and 2.2 g kg^{-1} total N and P, respectively, and about 50% water content. Manure, P and K were applied as basal fertilizers, while 40% of the N was applied as a basal dressing and 60% top-dressed on the wheat crop at the reviving growth stage.

In this study, rhizosphere soil was defined as the vegetated soil within the densely rooted portion of the soil profile, and bulk soil as the unvegetated soil immediately surrounding the root mat (Kourtev et al., 2002). Soil samples were collected at the wheat reproductive stage in early May 2010, then rhizosphere effects tend to be most pronounced (Cheng et al., 2003). The random sampling method was used to ensure representative sampling from the different treatments. One composite bulk or rhizosphere soil sample, consisting of 20 paired cores from rhizosphere or neighboring bulk soils, was collected from each treatment. The samples were immediately transported to the laboratory. Plants roots were removed by passing through a 2 mm mesh sieve, and the samples were then stored at room temperature for chemical analysis, at 4 °C for extracellular enzyme analysis, and at −70 °C for PLFA analysis (i.e. the soil was freeze-dried before the determination of PLFAs).

2.2. Chemical analysis

Soil pH was measured with a compound electrode (PE-10, Sartorious, Germany) using a soil to water ratio of 1:2.5. Soil organic C was determined by dichromate oxidation, and total N and total S by element analyzer (Elementar Analysensysteme GmbH, Germany). Ammonium N (NH_4^+-N) and nitrate N (NO_3^--N) contents were determined by extracting the soil with 0.01 M KCl solution (1:10, w/v) for 30 min, and determining NH_4^+ and NO_3^- concentrations by flow injection autoanalyzer (FLA star 5000 Analyzer, Foss, Denmark). Available P was determined by the Olsen method (Olsen and Sommers, 1982) and available K was analyzed by ammonium displacement of the exchangeable cations.

2.3. Extracellular enzyme activities

The activities of all extracellular enzymes except urease, phenol oxidase and peroxidase were measured using MUF-linked or AMC-linked model substrates yielding the highly fluorescent cleavage products 4-methylumbelliferyl (MUF) or 7-amino-4-methylcoumarin (AMC) upon hydrolysis (Deforest, 2009; Saiya-Cork et al., 2002; Wittmann et al., 2004) (Table 1). The method is very sensitive and allowed a high throughput analysis of enzymatic activities (Wittmann et