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Spatio-temporal patterns of enzyme activities after manure application reflect mechanisms of niche differentiation between plants and microorganisms

Shibin Liu [a,*], Bahar S. Razavi [b], Xu Su [c], Menuka Maharjan [b], Mohsen Zarebanadkouki [d], Evgenia Blagodatskaya [a], Yakov Kuzyakov [a,b,e]

[a] Department of Soil Science of Temperate Ecosystems, University of Göttingen, Göttingen, Germany
[b] Department of Agricultural Soil Science, University of Göttingen, Göttingen, Germany
[c] Key Laboratory of Education Ministry on Environments and Resources in Tibetan Plateau, Xining, China
[d] Division of Soil Hydrology, University of Göttingen, Göttingen, Germany
[e] Huazhong Agricultural University, Wuhan, China

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ABSTRACT

Manure is an important source of nutrients for plants and stimulates a wide range of enzyme-mediated microbial processes. Such stimulation, however, depends on manure distribution and the duration of its decomposition in soil. For the first time, we investigated the spatio-temporal patterns of enzyme activities as affected by manure application strategies: 1) Localized manure: manure application as a layer in the upper soil; 2) Homogenized manure: mixing manure throughout the soil; and 3) Control without manure. Tibetan barley was planted on soil managed with yak manure from the Tibetan Plateau. Soil zymography was used to visualize the two-dimensional distribution and dynamics of the activities of three enzymes responsible for cycling of carbon (β-glucosidase), nitrogen (N-acetylglucosaminidase) and phosphorus (phosphomonoesterase) over 45 days. The manure detritusphere increased enzyme activities relative to the control (which had only the rhizosphere effect of barley) and this stimulation lasted less than 45 days. Enzyme activities in the manure-induced hotspots were higher than on the barley rhizoplane, indicating that the detritusphere stimulated microbial activities more strongly than roots. Homogenized manure led to 3–29% higher enzyme activities than localized manure, but shoot and root biomass was respectively 3.1 and 6.7 times higher with localized manure application. Nutrients released by high enzyme activities within the whole soil volume will be efficiently trapped by microorganisms. In contrast, nutrients released from manure locally are in excess for microbial uptake and remain available for roots. Consequently, microorganisms were successful competitors for nutrients from homogeneous manure application, while plants benefited more from localized manure application. We conclude that localized manure application decreases competition for nutrients between the microbial community of manure and the roots, and thereby increases plant performance.

1. Introduction

Livestock manure application has been widely accepted as a sustainable management practice in agriculture, providing environmentally and agronomically sound outcomes (Risse et al., 2006; Brandjes et al., 1996; Scotti et al., 2015). Manure incorporation into soil forms a detritusphere abundant in organic carbon (OC) and nutrients (Moore et al., 2004). It is beneficial for improvement of soil quality and crop production (Butler et al., 2013; Calleja-Cervantes et al., 2015; Zaller and Köpke, 2004).

The application strategy is an important aspect of manure management (Webb et al., 2010; Thomsen, 2005). It affects soil-plant-microbial interactions by determining the locations of nutrients or altering soil properties (moisture, O_2 diffusion, bulk density) (Acosta-Martínez and Waldrip, 2014; Zhu et al., 2015). As a consequence, responses of plants and microorganisms vary depending on the manure application strategy. For instance, mixing of manure into soil increased soil microbial biomass (Lovell and

* Corresponding author.
E-mail address: sliu3@gwdg.de (S. Liu).

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Jarvis, 1996; Malik et al., 2013), but no response of soil microbial biomass was observed when manure pats were placed on the soil surface (Lovell and Jarvis, 1996; Cai et al., 2014). Although remarkable increases in plant production have been reported after either incorporating manure into soil (Malik et al., 2013) or broadcasting manure on the soil surface (Aarons et al., 2009; Matilla, 2006), a direct comparison of plant production under various manure application strategies is still lacking.

Enzymes, excreted by both plants and microbes, are early indicators of soil quality and the main mediators of organic matter decomposition (Nannipieri et al., 2007; Sinsabaugh et al., 2008). Assays of enzyme activities have been widely used to investigate the influence of manure application on soil nutrient cycling and microbial activities. Most studies observed significantly increased enzyme activities in soils amended with livestock manures (Liang et al., 2014; Calleja-Cervantes et al., 2015; Bell et al., 2006). However, the study of spatial and temporal responses of enzyme activities requires advanced visualization technology (Acosta-Martínez and Waldrip, 2014).

On the Tibetan Plateau, yaks (Bos grunniens) are one of the main species of livestock, and around 40% of their manure is used as fertilizer for cropland and pastures (FAO, 2003; Wang, 2009). However, the impact of yak manure application strategies on the growth of Tibetan barley – a staple crop – and on soil enzyme activities remains unknown. Such knowledge could lead to better manure application strategies. We used soil from the Tibetan Plateau for better consideration of local nutrient conditions and soil properties, and in the context of prevalent ecosystem degradation (Babel et al., 2014; Hafner et al., 2012).

Here we used direct soil zymography (Razavi et al., 2016) to investigate the impact of different yak manure application strategies on the growth of Tibetan barley (*Hordeum vulgare* L.) and on the temporal and spatial patterns of enzyme activities in Tibetan soil. We compared manure application strategies using three treatments (Fig. 1): 1) Localized manure: manure application as a layer in the upper soil; 2) Homogenized manure: mixing manure throughout the soil; and 3) No manure: a control without manure application. Our objectives were to investigate the effects of manure application strategy on plant shoot and root biomass and on the spatial and temporal patterns of soil enzyme activities. Direct soil zymography was used to visualize and quantify the spatial and temporal distribution of enzyme activity for the three enzymes: β-glucosidase, phosphomonoesterase and N-acetylglucosaminidase. β-glucosidase is responsible for catalyzing the hydrolysis of terminal 1,4-linked β-D-glucose residues from β-D-glucosides (German et al., 2011) and is involved in the carbon (C) cycle. Phosphomonoesterase, which catalyzes the hydrolysis of organic phosphorus (P) compounds to inorganic P (Eivazi and Tabatabai, 1977; Malcolm, 1983), is involved in the P cycle. N-acetylglucosaminidase (chitinase), which accomplishes the decomposition of chitin to yield low molecular weight chitooligomers (Hamid et al., 2013), is responsible for C- and nitrogen (N) -acquisition.

The considerable addition of labile organic compounds and nutrients in manure are expected to greatly influence plant and microorganism activities, and therefore soil enzyme activities. We hypothesized - H1: weaker enzyme activities at the root-soil interface as compared with a strong increase of enzyme activities in the manure-induced detritusphere; H2: stronger stimulation of plant growth by the homogenized manure application strategy.

2. Materials and methods

2.1. Soil and yak dung sampling

Soil was sampled at the research station "*Kobresia* Ecosystem Monitoring Area" (KEMA) (31°16′45″N 92°59′37″E, 4410 m a.s.l.), which was established by Prof. Georg Miehe with the support of the VW foundation, and which now belongs to the Tibet University and the Institute of Tibetan Plateau Research in Nagqu. The soil was classified as a *Stagnic Eutric Cambisol* (*Humic*) (WRB, 2014) with a texture of 50% sand, 33% silt and 17% clay. The pH value (H_2O) was 6.9 ± 0.03 and soil bulk density was 0.92 g cm^{-3}. Yak dung was collected from Nangqian town, Yu Shu Prefecture (32°04′N, 96°31′E, 3600 m a.s.l.). Before being sampled, dung was piled and composted in the field.

In total, 10 soil core samples (25 cm deep, 5 cm diameter) were taken within an area of ca. 100 m^2. All the samples were hand-mixed and roots and stones were removed. The composite soil and composted yak dung samples were stored in ziplock bags at 4 °C, transported to the laboratory of the University of Göttingen and passed through a 2 mm sieve in preparation for incubation. Daily mean temperature during the sampling month ranged from 3.2 °C to 21.3 °C, so the temperature used for transportation was not uncommon and would not strongly affect soil and manure characteristics.

Additional soil and dung samples were oven-dried at 60 °C for 48 h to measure carbon (C) and nitrogen (N) content. Initial water content was measured by oven-drying samples at 105 °C. Soil C and N contents were 3.4 ± 0.11% and 0.3 ± 0.01%, respectively. The yak dung contained 37 ± 0.3% C and 1.3 ± 0.04% N.

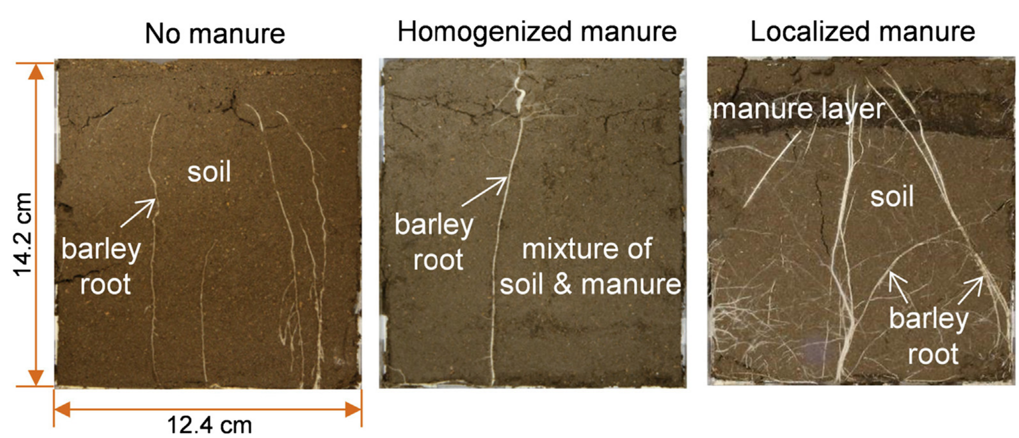

Fig. 1. Rhizoboxes with barley growing under three manure application strategies: No manure (left), manure homogenized with the whole soil (middle), and manure localized in the soil layer between 1.0 and 2.5 cm below the soil surface (right).