# Responses of enzymatic activities within soil aggregates to 9-year nitrogen and water addition in a semi-arid grassland

CrossMark

Ruzhen Wang [a, f], Maxim Dorodnikov [b], Shan Yang [a, c], Yongyong Zhang [a, f], Timothy R. Filley [d], Ronald F. Turco [e], Yuge Zhang [c], Zhuwen Xu [a], Hui Li [a], Yong Jiang [a, *]

[a] State Key Laboratory of Forest and Soil Ecology, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China
[b] Soil Science of Temperate Ecosystems, Büsgen-Institute, Georg August University of Göttingen, Büsgenweg 2, 37077 Göttingen, Germany
[c] Key Laboratory of Regional Environment and Eco-remediation, College of Environment, Shenyang University, Shenyang 110044, China
[d] Department of Earth, Atmospheric, and Planetary Sciences, Purdue University, West Lafayette, IN 47907, USA
[e] Department of Agronomy, Purdue University, West Lafayette, IN 47907, USA
[f] University of Chinese Academy of Sciences, Beijing 100049, China

## ARTICLE INFO

## ABSTRACT

Soil microorganisms secrete enzymes used to metabolize carbon (C), nitrogen (N), and phosphorus (P) from the organic materials typically found in soil. Because of the connection with the active microbial biomass, soil enzyme activities can be used to investigate microbial nutrient cycling including the microbial response to environmental changes, transformation rates and to address the location of the most active biomass. In a 9-year field study on global change scenarios related to increasing N inputs (ambient to 15 g N m$^{-2}$ yr$^{-1}$) and precipitation (ambient to 180 mm yr$^{-1}$), we tested the activities of soil β-glucosidase (BG), N-acetyl-glucosaminidase (NAG) and acid phosphomonoesterase (PME) for three soil aggregate classes: large macroaggregates (>2000 μm), small macroaggregates (250–2000 μm) and microaggregates (<250 μm). Results showed higher BG and PME activities in micro-vs. small macroaggregates whereas the highest NAG activity was found in the large macroaggregates. This distribution of enzyme activity suggests a higher contribution of fast-growing microorganisms in the micro-compared with the macroaggregates size fractions. The responses of BG and PME were different from NAG activity under N addition, as BG and PME decreased as much as 47.1% and 36.3%, respectively, while the NAG increased by as much as 80.8%, which could imply better adaption of fungi than bacteria to lower soil pH conditions developed under increased N. Significant increases in BG and PME activities by as much as 103.4 and 75.4%, respectively, were found under water addition. Lower ratio of BG:NAG and higher NAG:PME underlined enhanced microbial N limitation relative to both C and P, suggesting the repression of microbial activity and the accompanied decline in their ability to compete for N with plants and/or the accelerated proliferation of soil fungi under elevated N inputs. We conclude that changes in microbial activities under increased N input and greater water availability in arid- and semi-arid grassland ecosystems where NPP is co-limited by N and water may result in substantial redistribution of microbial activity in different-sized soil particles. This shift will influence the stability of SOM in the soil aggregates and the nutrient limitation of soil biota.

## 1. Introduction

Extracellular enzymes are secreted by soil microorganisms to mineralize organic carbon (C), nitrogen (N), and phosphorus (P) from soil organic matter (Waring et al., 2014). Extracellular activities are distinct from intracellular ones as they can be stabilized by abiotic soil components (Dilly and Nannipieri, 1998). Measured enzyme activities represent the apparent catalytic history of a soil as continuously modified by soil microorganism in response to environmental changes (Dilly and Nannipieri, 2001). As a result, enzyme activities can be used to assess microbial nutrient demands (Schimel and Weintraub, 2003; Moorhead and Sinsabaugh, 2006) and used to formulate an ecosystem response index that reflect environmental changes (Ajwa et al., 1999; Sinsabaugh et al., 2008). For example, β-glucosidase (BG) has been used to assess the microbial response to long-term N amendments in a tall-grass prairie

* Corresponding author. Tel.: +86 24 83970902; fax: +86 24 83970300.
E-mail address: jiangyong@iae.ac.cn (Y. Jiang).

soil (Ajwa et al., 1999) and N-acetyl-glucosaminidase (NAG) has been utilized to quantify N-limitation effecting woody plant encroachment into grasslands (Creamer et al., 2013). Enzymatic process stoichiometry is suggested as a means to understand the C and N limitations in soil processes as it is demonstrated that soil enzymatic activities and stoichiometry are related to substrate availability, soil pH, and climatic factors (e.g., precipitation and temperature) (Sinsabaugh et al., 2008, 2009; Waring et al., 2013). However, the effects of environmental factors, such as elevated N inputs and precipitation on enzymatic stoichiometry are unclear. Various global change scenarios have suggested that increased inputs of reactive N from fertilization and fossil fuel combustion and altered precipitation regimes will become common (Knapp et al., 2002; Liu et al., 2009, 2013). As soil enzyme activities are sensitive to ecosystem fluctuations, they can serve as indicators of various responses of the plant-soil system to changes in N deposition (Sinsabaugh et al., 2005), elevated atmospheric $CO_2$ (Dorodnikov et al., 2009a,b), and precipitation intensity (Henry et al., 2005; Bardgett et al., 2008; Pendall et al., 2008).

Clearly, nitrogen and water availability are two driving factors affecting grassland net primary productivity (NPP) (Xu et al., 2012), especially in semi-arid grasslands where evaporation greatly exceeds annual precipitation inputs (Heisler-White et al., 2008). Recent grassland-related field studies in Inner Mongolia suggested a plant NPP of about 1.5 tons ha$^{-1}$ is both N- and water-limited, because N addition above 5.25–17.5 g N m$^{-2}$ yr$^{-1}$ of background increased NPP by 13%–62 % (Bai et al., 2010), while water addition increased above- and belowground NPP by 32.9% and 38.3%, respectively (Xu et al., 2010). However, soil microorganisms are not limited by the same factors that limit plant systems (Hobbie et al., 2005; Wei et al., 2013). For example, Wei et al. (2013) reported different N saturation levels (threshold levels for N demand) for plants and soil microorganism highlighting that microbes can be limited by C or P while plants are N limited (Treseder, 2008). Additionally, reduction in both the size and activity of soil microbial biomass were shown under higher N availability in temperate grasslands (Gutknecht et al., 2012; Wei et al., 2013), which indicates microorganisms are not always limited by N. On the other hand, positive effects of added N were also observed (Zeglin et al., 2007; Keeler et al., 2009). Keeler et al. (2009) found N addition to increase the activity of phosphatase and cellobiohydrolase by 13% and 17%, respectively. Similar findings were also reported by Zeglin et al. (2007) where N increased both cellulolytic activities (BG and cellobiohydrolase) and phosphatase activity. Other studies showed that added water in grassland ecosystems stimulated (Zhou et al., 2013) or suppressed microbial activity (Henry et al., 2005) depending on the study site. For instance, increased water availability resulted in increases of NAG, leucine aminopeptidase, and alkaline phosphomonoesterase (PME) in an Inner Mongolia grassland (Zhou et al., 2013), while water addition resulted in decreases of BG, NAG, and PME in a California grassland soil (Henry et al., 2005).

Aggregate structure can affect microbial activities as fluxes of water and oxygen (Six et al., 2004) and accessibility of SOM will differ between aggregate-size classes (Jastrow et al., 2007). Jastrow et al. (2007) report most labile SOM is concentrated in macroaggregates and more recalcitrant, or less accessible SOM is resident in microaggregates resulting in overall higher enzyme activities in macro-vs. microaggregates (Dorodnikov et al., 2009b). The study of soil microbial enzyme activities on an aggregate level could provide insight into soil C and N cycling in response to increased N input and precipitation.

The objectives of this study were to examine the effects of elevated N inputs and precipitation intensity on the distribution and activity of C-, N-, and P-acquiring enzymes by evaluating aggregate size fractions for soils collected from the semi-arid grasslands of Inner Mongolia, China. A prior field manipulation experiments, had demonstrated significant increase of NPP in response to four 4-year N and water addition (Xu et al., 2010) and SOC in response to 7-year water addition (Wang et al., 2014). We hypothesized that (i) microbial biomass and enzyme activities would increase in macroaggregates because of presumably higher amount of labile SOM; (ii) N- acquiring enzymes would respond to N additions in a way that is different from C- and P-acquiring enzymes because N addition would decrease the substrate C:N ratio and increase the N:P ratio; and (iii) increasing moisture inputs for ecosystems under water limitation should stimulate microbial activity resulting in higher overall enzyme production for C-, N-, and P-acquisition. As N amendment may potentially cause C and P limitation, we predict that the ratio of β-glucosidase to N-acetyl-glucosaminidase will increase while N-acetyl-glucosaminidase to phosphomonoesterase ratio will decrease under higher N availability.

## 2. Materials and methods

### 2.1. Field site and experimental design

The study site is located in Duolun County, Inner Mongolia in northern China (116°17′ E and 42°02′ N, elevation 1324 m a.s.l.). The mean annual temperature is 2.1 °C with mean monthly temperature ranging from −17.5 °C in January to 18.9 °C in July, and the mean annual precipitation is 379.4 mm with approximately 86% occurring from May to September. The plant community at the site is a typical temperate grassland dominated by prairie sagewort (*Artemisia frigida* Willd.), wheatgrass (*Agropyron cristatum* Gaertn.), and needlegrass (*Stipa krylovii* Roshev.). The soil type is classified as Haplic Calcisols according to the FAO classification with 63% sand, 20% silt, and 17% clay, respectively (Liu et al., 2009).

In April 2005, a split-plot experiment design was applied to the site. Twelve 8 m × 8 m plots were established in each treatment block (107 m × 8 m) with 1 m buffer zone between any two adjacent plots; each block was replicated seven times. The blocks were divided into two main plots based on water treatment (ambient precipitation and 180 mm of water addition) and then each main plot was divided into six subplots. The 180 mm of water addition is an approximately 50% increase above mean annual precipitation based on meteorological record for the site (Xu et al., 2012). This experiment is part of an on-going project designed to investigate the effect of increased N and water on ecosystem responses in the Inner Mongolia grassland (Xu et al., 2010, 2012). The N addition plots were randomly assigned to subplots within each main plot. The water addition plots received 15 mm of precipitation weekly by sprinkling irrigation during the growing season (12 consecutive weeks from June to August). Nitrogen (in the form of urea) was applied at four levels: 0 (CK), 5 g N m$^{-2}$ yr$^{-1}$ (N$_5$), 10 g N m$^{-2}$ yr$^{-1}$ (N$_{10}$), and 15 g N m$^{-2}$ yr$^{-1}$ (N$_{15}$) in four of six subplots, half of which was applied in early May and the other half in late June from 2005 to 2013. Background N inputs (atmospheric deposition plus fertilizer application) in this area are about 5 g m$^{-2}$ yr$^{-1}$ so this manipulation represents a 5–10 g m$^{-2}$ yr$^{-1}$ increase in N addition.

### 2.2. Soil aggregate-size fractionation and other soil physicochemical properties

In September 2013, a composite soil sample from the top 0–10 cm soil layer was taken from five randomly selected locations at each plot from four out of seven blocks in both the N and water treatment main plots using a 5-cm diameter corer. Fresh soil