The effects of a 9-year nitrogen and water addition on soil aggregate phosphorus and sulfur availability in a semi-arid grassland

Ruzhen Wang [a], Courtney A. Creamer [b], Xue Wang [a,c], Peng He [a,c], Zhuwen Xu [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] CSIRO Sustainable Agriculture Flagship, Glen Osmond, SA 5064, Australia
[c] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

ABSTRACT

Previous studies have demonstrated that higher nitrogen (N) and water availability affect both above- and below-ground communities, soil carbon and N pools, and microbial activity in semi-arid grasslands of Inner Mongolia. However, how soil phosphorus (P) and sulfur (S) pools, and related soil enzyme activities (as indicators of P and S cycles) respond to long-term N and water addition has still remained unclear. Since 2005, a field experiment with urea and water amendments has been conducted to examine their effects on total and available P and S concentrations and alkaline phosphomonoesterase (PME) and aryl-sulfatase (ArS) activities in three soil aggregate fractions: large macroaggregates (>2 mm), small macroaggregates (0.25–2 mm), and microaggregates (<0.25 mm) in an Inner Mongolia semi-arid grassland. Normalized to aggregate mass, microaggregates retained the highest total P and S concentrations. Both N and water additions increased the available P (by up to 84.5%) and the available S (by up to 150%) in the soil aggregate fractions. Soil acidification, as a result of the N addition, decreased both alkaline PME and ArS activities by up to 62.9% and 39.6%, respectively, while the water addition increased their activities. Our observations revealed that soil acidification (under the N addition) and elevated enzyme activity (under the water addition) played important roles in the levels of soil available P and S. The depression of P- and S-acquiring enzymes with soil acidification may decrease P and S availability, potentially impacting ecosystem processes and limiting the restoration of these grassland systems. The water addition was shown to be a more effective practice than the urea amendment for improving soil structure, supplying available P and S, and maintaining the sustainability of this semi-arid grassland.

1. Introduction

Increasing nitrogen (N) inputs and changing precipitation regimes are two important and interacting factors influencing terrestrial ecosystems under global change scenarios (Bai et al., 2010; Zhang et al., 2013), especially for semi-arid grasslands which are co-limited by N and water. In China, over 78% of grasslands are distributed in the northern temperate and semi-arid zones, like Inner Mongolia (Kang et al., 2007). Previous studies in these semi-arid grasslands have demonstrated that N and water addition can affect net primary production (NPP) (Bai et al., 2010), species turnover, plant community composition (Xu et al., 2012), and even soil

organic carbon (C) content and microbial community composition (Zhang et al., 2013; Zhou et al., 2013a,b; Wang et al., 2014). Nitrogen fertilization can result in grassland acidification (Yang et al., 2012; Wang et al., 2014), changes in the microbial community composition (Wei et al., 2013), and reductions in microbial activity (Wang et al., 2014). In contrast, water addition can increase microbial activity or mediate suppressive effects of N addition, and can influence the abundance, richness, and composition of bacterial communities (Zhou et al., 2013a; Wang et al., 2014; Zhang et al., 2014). Though numerous studies have concentrated on responses of above- and below-ground communities to N and water addition in these areas, little is known about the effects of N and water amendment on soil phosphorus (P) and sulfur (S) dynamics.

The turnover of soil carbon and nutrients is influenced by P and S availability (Mooshammer et al., 2012; Manzoni et al., 2010; Parton et al., 1988). Nitrogen addition can reduce the sorption capacity

of soil P ions (Stroia et al., 2011) which can potentially increase P availability, particularly in calcareous soils (Tunesi et al., 1999). Observations of the long-term effects of N fertilization on P availability have produced contrasting results, mainly because high N inputs may enhance P uptake (in the form of $H_2PO_4^-$ and HPO_4^{2-}) by plants (Olander and Vitousek, 2000), but also reduce phosphatase activity due to soil acidification (Wang et al., 2014). In this case, the amount of phosphate ions in soil solution depends upon the balance between sorption capacity of PO_4^{3-} to soil minerals and phosphate ions absorbed by plants (Tunesi et al., 1999). Under N addition, mineralization of soil organic P might decrease due to phosphatase suppression (Olander and Vitousek, 2000). Under elevated water regimes, an increase in soil moisture enhances P availability for uptake by plants and microbes as the diffusivity of P increases (Lambers et al., 2006). Similarly, N fertilization can also intensify the mineralization of C-bonded S resulting in higher S availability (in the form of SO_4-S) (De Bona and Monteiro, 2010). How soil available S responds to water addition is still unclear and related studies are rarely seen.

As soil P and S cycling is largely associated with soil organic matter (SOM) dynamics, factors controlling SOM stabilization will impact soil P and S storage and influence biologically-associated P and S pools in ecosystems (Fonte et al., 2014; McGill and Cole, 1981). Soil aggregation, driven by binding agents such as SOM, arbuscular mycorrhizal fungi, and roots, has been long considered as a key factor in providing physical protection for SOM (Jastrow et al., 1998). In particular, microaggregates (<0.25 mm) are thought to play a dominant role in SOM storage (O'Brien and Jastrow, 2013). Macroaggregates (>0.25 mm) are responsible for the early stages of SOM protection, which provide a preferential site for the formation and stabilization of C within microaggregates (Six et al., 2004). Though many studies have concentrated on soil C and N dynamics (Gunina and Kuzyakov, 2014; O'Brien and Jastrow, 2013; Wilson et al., 2009) or microbial community composition and activity (Dorodnikov et al., 2009) within soil aggregates, studies concerning aggregate-scale P and S storage and dynamics are still rarely seen, especially in these nutrient-poor grasslands under global change scenarios of enhanced N input and changing precipitation regimes.

Microbial extracellular enzymes play a controlling role over SOM decomposition (Henry et al., 2005) and are widely used to indicate responses of belowground processes to exogenous disturbances (Sinsabaugh et al., 2002). The activities of phosphatases are responsible for organic P hydrolysis and available P supply to plants (Wei et al., 2014). Aryl-sulfatase is secreted to release soil available S from organic S (mainly ester sulfates) for plant growth (Tabatabai, 1994). Previous studies have shown that N fertilization increases acid and alkaline phosphomonoesterases (PME) activities while soil P availability is negatively correlated with acid PME (Colvan et al., 2001). Also, aryl-sulfatase activity can decrease under conditions of soil acidification (Brockett et al., 2012). However, studies of how these enzyme activities respond to N fertilization in presence of elevated water inputs are still rarely seen.

In previous works we showed that a prolonged N fertilization with urea and water amendment enhanced soil aggregate stability (Wang et al., 2015a,b). In this study we hypothesized that microaggregates would have higher total P and S contents when normalized to aggregate mass due to better SOM protection, and that both N and water additions would increase the availability of P and S in the soil. We also expected that soil aggregate P- and S-cycling enzyme activities would decrease under elevated N inputs due to a suppressive effect of lower soil pH, while enzyme activities would increase under higher water availability. We tested these hypotheses by analyzing soils from a nine-year long field trial which has important implications for the sustainability of these vulnerable and essential ecosystems.

2. Materials and methods

2.1. Study sites and experimental design

In April 2005, a field trial was established at the south of Duolun County of Inner Mongolia, China (42°02′27″ N, 116°17′59″ E, elevation 1324 m a.s.l.) on a sandy loam soil (Calcisorthic Aridisol, in the U.S. Soil Taxonomy classification). The trial area has undergone severe degradation due to overgrazing during the last 50 years, but the experimental plots of the present study were fenced in 2000 to investigate the impacts of grassland management practices (N fertilization and irrigation) on plant diversity and soil properties (Wang et al., 2014). The site has a semi-arid climate with mean annual precipitation of 379 mm and mean annual temperature of 2.1 °C. The steppe is dominated by *Stipa krylovii* (C₃ grass), *Agropyron cristatum* (C₃ grass), and *Artemisia frigida* (C₃ forb).

Treatments were established on 8 m × 8 m plots arranged in a split-plot design (water and N addition) with seven replicates per treatment and 1 m buffer zone between any two adjacent plots. Each treatment block was divided into two main plots where water treatments (ambient precipitation and ambient plus water addition) were assigned. As a weekly 15-mm event, a total of 180 mm of additional water (+50% of mean annual precipitation) was sprinkled during growing season from June to August. Each main plot was divided into six subplots where four were randomly treated with one of four N addition 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}). Nitrogen was added as urea pellets with half applied in early May and the other half in late June. This study 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., 2012).

2.2. Soil sampling and aggregate isolation

In September 2013 (i.e. after 9 years of treatments), five randomly placed soil cores (0–10 cm) were taken from four replicate plots of the N and water treatments, using a 10-cm-deep corer (5 cm in diameter). Fresh soil samples were stored at 4 °C in hard plastic containers (to maintain their primary structures) during transportation to the laboratory. To minimize the impact on microbial activity and nutrient availability, aggregates were isolated by a dry-sieving method as described by Wang et al. (2015a). Soil samples were isolated into three aggregate size classes: >2 mm (large macroaggregates class), 0.25–2 mm (small macroaggregates class), and <0.25 mm (microaggregates class). No correction was made for sand (e.g. Elliott and Cambardella, 1991) as sand grains can be embedded in soil aggregates. Although this prevents direct comparisons of mineral-associated OM between aggregates, it allows for the potential that OM coatings on sand grains may be important for SOM turnover, and that sand may be an important part of the aggregate structure. This was preferred to examine whole aggregate (including sand) P and S dynamics. However, as a result differences in aggregate sizes cannot be attributed solely to mineral associations. The chemical characteristics of the soil aggregates (0–10 cm) are given in Table 1 (Wang et al., 2015a,b).

2.3. Analyses of soil chemical and biological parameters

Soil pH was determined in a 1:5 (w/v) soil-water extract of soil aggregate samples by a PHS-3G digital pH meter (Precision and Scientific Crop., Shanghai, China).

Total P in the aggregate fractions was determined by perchloric acid ($HClO_4$) digestion (Sommers and Nelson, 1972) and analyzed by the molybdenum blue colorimetric method (Murphy and Riley, 1962). Briefly, 0.5 g of the air-dried soil (<100-mesh) was weighed into separate digestion tubes and mixed with 7.5 ml of 70% $HClO_4$.