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# Seasonal changes of soil microbial C, N, P and associated nutrient dynamics in a semiarid grassland of north China

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ABSTRACT

Semiarid grasslands are widely distributed in northern China and characterized by marked seasonality. While the role of soil microbes in nutrient cycling is known to be crucial, the nutrient dynamics in relation to changes and turnover of microbial pools over the growth season in semiarid grasslands, are not well understood. In this study, three grasslands with long-term traditional managements (enclosure from sheep grazing for 31 or 18 years, or continuous free overgrazing) were selected to investigate the seasonal fluctuations of microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP). In addition we calculated the turnover rates and fluxes of soil microbial biomass based on their seasonal fluctuations. Plant biomass and N, P uptakes were also assessed to reveal a potential relationship between plant and microbial nutrient pools. We found in this semiarid ecosystem approximately two times lower C:P and N:P ratios in microbial biomass (25:1 and 3:1, respectively) compared with global analysis (46:1 and 6:1, respectively). Enclosure from grazing increased MBC and MBN, while the change patterns of microbial pools were affected by season but not pasture management. Consequently, the turnover rates of microbial biomass as calculated from the seasonal fluctuations were similar in all treatments (around 1.5 $year^{-1}$ for MBC and MBN, 3 $year^{-1}$ for MBP). Lower mean stock in soil $K_2SO_4$-extractable N but similar in MBN compared with total plant N uptake were observed in all treatments, suggesting N deficiency in this region and the vital role soil microbes play as a stable nutrient pool for plant uptake. In contrast, both $NaHCO_3$-extractable P and MBP stocks were much higher than total plant P uptake, suggesting no P deficiency under current N status.

## 1. Introduction

Soil microbes play an essential role in the main biogeochemical transformation of organic matter and in soil fertility (Jenkinson and Ladd, 1981). During the mineralization process, an important fraction of the C, N and P in the decomposing residues is immobilized in the microbial biomass as part of their cellular constituents, and then released upon microbial death (Anderson and Domsch, 1980; Jonasson et al., 1996). The soil microbial biomass therefore acts as both a sink and a source of labile nutrient pools during the turnover (Griffiths et al., 2012).

Generally the carbon-to-nutrient ratio determines whether nutrients are immobilized in the microbial biomass or mineralized to become available for plant uptake. Thus, the stoichiometry of C, N, and P is a powerful tool to decipher their coupling mechanisms and nutrient limitation in terrestrial ecosystems (Ågren et al., 2012; Aponte et al., 2010; Kirkby et al., 2011; Ostrowska and Porębska, 2015). Many studies have shown that the C:N:P ratios of soil and the soil microbial biomass were constrained under near optimum soil conditions (Bing et al., 2016; Cleveland and Liptzin, 2007; Griffiths et al., 2012;). However, ratios in soil microbial biomass vary with species growth rate (Hillebrand et al., 2013), trophic level, and environmental parameters (Guignard et al., 2017). Soil microbes are very sensitive to the changes of environmental conditions, such as the availability and limitation of the soil substrate (e.g. C, N and P fertility management) (Cruz et al., 2009; Wang et al., 2010), temperature and moisture (Fang and Moncrief, 2001; Hamel et al., 2006), competition between plants and microorganisms for nutrients (Rousk et al., 2007), and inputs of organic matter from above- and belowground plant residues (Chen et al., 2003). As a result, soil microbial biomass is likely to present obvious fluctuations during the growth season. Thus, while general ratios have been described, it is incentive to further understand how the microbial C:N:P stoichiometry changes seasonally.

Furthermore, microbial turnover rates can be estimated by dividing

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the sum of losses in fluctuations by the average microbial biomass (McGill et al., 1986). Since this concept is based on net changes of microbial biomass and the turnover occurs even if biomass remains unchanged overtime, it is a minimum estimate of microbial turnover and would need to be verified by tracer data (Harden and Joergensen, 2000; Liebisch et al., 2014). However, the McGill approach has been widely used in field studies (Liebisch et al., 2014; McGill et al., 1986; von Lützow and Ottow, 1994) due to problems of applying radioisotopes in tracer-based approaches (e.g. $^{32}P$ or $^{33}P$ dilution experiments). Additionally, a qualitative comparison to the McGill approach across different ecosystems would be valuable (Liebisch et al., 2014; Oberson and Joner, 2005). Current knowledge suggests that microbial turnover time likely increases in soils with poor nutrient condition (Oberson and Joner, 2005). The reason for this seems to be that the efficiency of internal element recycling by microorganisms increases with decreasing element availability (Spohn and Widdig, 2017). Microbial biomass N (MBN) and P (MBP) fluxes derived from turnover account for much of the plant N and P uptake (Bünemann et al., 2012; He et al., 2002).

Semiarid grassland ecosystems are subjected to a marked seasonality and characterized by distinct fluctuation of temperature and moisture over a year. Due to overgrazing in the past decades, grasslands in northern China have been suffering from serious degradation (Liu et al., 2016). Sitters and Venterink (2015) concluded the main effects of grazing on soil and plants to be the mismatch of C:N:P stoichiometry, litter quality and soil compaction, etc. Enclosure of area to exclude grazing animals for years is widely used as a rehabilitation and reconstruction method for degenerated grasslands (Aerts et al., 2004; Armitage et al., 2012; Hüseyin et al., 2007). A previous study carried out in this area has shown that enclosure from grazing increased MBC and MBN (Liu et al., 2016). An overall understanding of seasonal changes and stoichiometry of MBC, MBN and MBP under these grassland management regimes (grazing and different restoration phases) in this ecosystem would be very helpful to predict and manage grasslands under a changing climate.

Our main objectives were (1) to investigate the seasonal dynamics and (2) to estimate the turnover of soil MBC, MBN, and MBP in semiarid grasslands under continuous grazing and enclosure from grazing for 18 and 31 years. We hypothesized that the sizes of soil microbial pools in enclosure treatments are larger during the growth season and microbial turnovers are faster compared with the grazing treatment. To reveal a potential relationship between plants and microorganisms, we also monitored plant N, P uptake. Climatic conditions (air temperature, precipitation and soil moisture) were monitored to identify the factors underlying the seasonal dynamics of soil C, N, and P pools.

## 2. Material and methods

### 2.1. Description of study sites

The research site is in a typical steppe ecosystem in northern China, located near the Inner Mongolia Grassland Ecosystem Research Station of the Chinese Academy of Sciences (N 43°38′, E 116°42′; 1200 m above sea level). The region has a temperate semiarid continental climate with an annual average temperature of 3.5 °C, annual mean precipitation of 280–350 mm, and annual evaporation of 4–5 times that of the precipitation. The frost-free period is about 90 days. The soil is described as a dark chestnut soil (Chinese classification) or Calcic-Orthic Aridisol (Calcic Chernozem according to ISSS Working Group RB 1998). *Leymus chinensis* and *Stipa grandis* were the typical original pasture species in the region.

### 2.2. Field experimental design

The experimental site is composed of three large paddocks that are adjacent to each other, including two enclosure paddocks and one grazing paddock. Up to the year of sampling, two enclosure paddocks were excluded from sheep grazing for 31 years (since 1983, E83) and 18 years (since 1996, E96), respectively. A degraded paddock with continuous free grazing (FG) at about 9 sheep units $ha^{-1}$ $year^{-1}$ was used as a control. In order to decrease spatial variations and soil heterogeneity and to better manage the experiment, we first determined and fenced the sampling area at each paddock (80 × 200 m for both E83 and E96, 20 × 200 m for FG). And then we randomly chose three representative sub-plots in each sampling area (20 × 150 m for both E83 and E96, 20 × 50 m for FG, respectively). The distance between sub-plots in each sampling area was at least 5 m apart.

The grassland area had never received fertilizers and never been subjected to mow, the plant residues were naturally returned to the field as inputs for the next growth seasons. Long-term differences in grazing management resulted in different development of vegetation. Based on data of the maximum-biomass period in 2014, the order of biomass in E83 experimental plot is *S. grandis* (53%), *Agropyron michnoi* (13%), *Kochia prostrata* (8%), *Carex korshinskyi* (8%), *Cleistogenes squarrosa* (6%), and *Achnatherum sibiricum* (5%). In E96, the order is *S. grandis* (46%), *L. chinensis* (20%), *A. michnoi* (10%), *K. prostrata* (9%), *C. korshinskyi* (4%), and *C. squarrosa* (3%). In FG, the order is *S. grandis* (51%), *C. korshinskyi* (10%), *C. squarrosa* (7%), *Iris lactea* var. *chinensis* (7%), *A. michnoi* (5%), and *L. chinensis* (5%). No legume was found at all three experimental sites. Long-term overgrazing of FG led to emergence of *Chenopodiaceae* and *Setaria viridis*. The full description of soil physicochemical characteristics is shown in Table 1.

### 2.3. Climatic data

For the study of the response of soil microbes to climatic changes under long-term management, we selected the daily mean climatic data in this region as reference, including daily average air temperatures and precipitation. The municipal meteorological station provided the data.

### 2.4. Soil sampling and analysis

The soils were sampled nine times, on the 25th of May, 10th of June, 3rd of July, 22nd of July, 14th of Aug, 3rd of Sep, 24th of Sep and 16th of Oct 2014, and 25th of May 2015, at E83, E96 and FG during the experimental period, which completely covered both the dry and rainy

**Table 1**
Soil physicochemical characteristics of surface layer (0–20 cm) in grassland enclosed from grazing since 1983 (E83), or since 1996 (E96), or with continuously free grazing (FG).

| Treatment | Bulk density ($g\ cm^{-3}$) | pH | Organic matter ($g\ kg^{-1}$) | Total nitrogen ($g\ kg^{-1}$) | Total phosphorus ($g\ kg^{-1}$) | Organicphosphorus ($g\ kg^{-1}$) | Water holding capacity ($g\ g^{-1}$) | Particle distribution (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | Sand | Silt | Clay |
| E83 | $1.20 \pm 0.08^{c}$ | $7.19 \pm 0.05^{b}$ | $30.16 \pm 1.51^{a}$ | $1.57 \pm 0.11^{a}$ | $0.33 \pm 0.01^{a}$ | $0.15 \pm 0.00^{a}$ | $0.51 \pm 0.01^{a}$ | 80.0 ± 1.8 | 11.9 ± 1.0 | 8.2 ± 1.0 |
| E96 | $1.36 \pm 0.14^{b}$ | $7.28 \pm 0.07^{ab}$ | $29.23 \pm 1.63^{a}$ | $1.61 \pm 0.13^{a}$ | $0.32 \pm 0.02^{a}$ | $0.14 \pm 0.01^{ab}$ | $0.47 \pm 0.01^{b}$ | 80.0 ± 4.8 | 11.9 ± 3.8 | 8.2 ± 1.0 |
| FG | $1.51 \pm 0.09^{a}$ | $7.29 \pm 0.06^{a}$ | $22.46 \pm 0.69^{b}$ | $1.16 \pm 0.06^{b}$ | $0.30 \pm 0.00^{b}$ | $0.13 \pm 0.01^{b}$ | $0.41 \pm 0.02^{c}$ | 84.8 ± 2.3 | 10.0 ± 0.9 | 5.2 ± 2.1 |

Means ± standard deviations. Different letters along the column indicate significant differences between mean values of each parameter among different management regimes at $P < 0.05$.