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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 ± 0.08^{c}	7.19 ± 0.05^{b}	30.16 ± 1.51^{a}	1.57 ± 0.11^{a}	0.33 ± 0.01^{a}	0.15 ± 0.00^{a}	0.51 ± 0.01^{a}	80.0 ± 1.8	11.9 ± 1.0	8.2 ± 1.0
E96	1.36 ± 0.14^{b}	7.28 ± 0.07^{ab}	29.23 ± 1.63^{a}	1.61 ± 0.13^{a}	0.32 ± 0.02^{a}	0.14 ± 0.01^{ab}	0.47 ± 0.01^{b}	80.0 ± 4.8	11.9 ± 3.8	8.2 ± 1.0
FG	1.51 ± 0.09^{a}	7.29 ± 0.06^{a}	22.46 ± 0.69^{b}	1.16 ± 0.06^{b}	0.30 ± 0.00^{b}	0.13 ± 0.01^{b}	0.41 ± 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 $P < 0.05$.

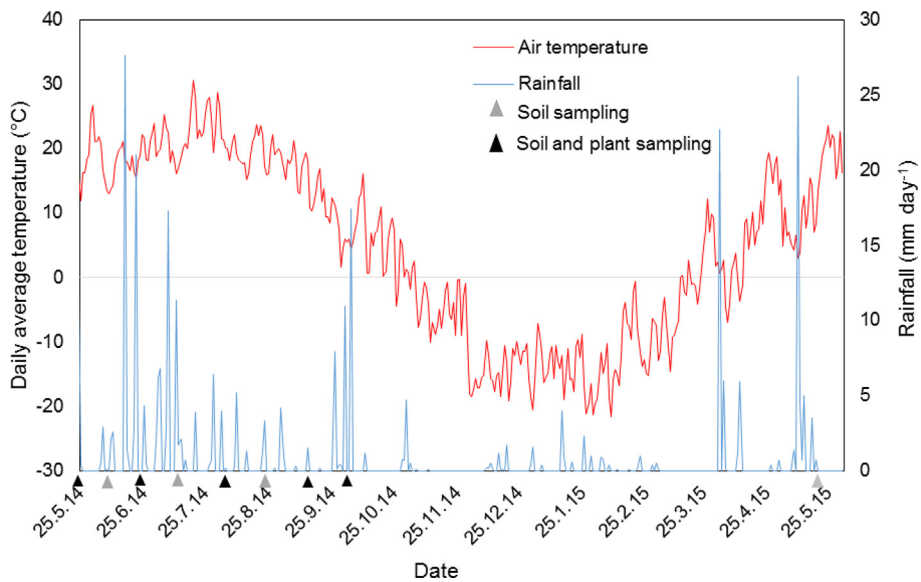

Fig. 1. Daily air temperatures, daily rainfall volumes, and soil and plant sampling dates. We used data for daily air temperatures and daily rainfall volumes from May 2014 to May 2015.

seasons (see Fig. 1). At each sampling, fifteen randomly distributed soil cores of 7-cm diameter and 20-cm depth were taken in each sub-plot (three replicates for each treatment), and then combined and mixed to create one composite sample. We chose 20-cm surface samples because we mainly focused on the soil microbial biomass dynamics in the present study. In addition, we did a vegetation investigation before sampling in the studied region, and found that the 20-cm depth is the area where the vast majority of plant roots distributed.

In the laboratory, coarse fresh plant debris were removed by passing through a sieve of mesh pore size of 4 mm and moist soil was then stored for a few days at 4 °C. A portion of soil was air dried and passed through a sieve of mesh pore size of 2 mm before the determination of physicochemical characteristics. Soil pH was determined in a water/soil suspension with a mass-volume ratio of 1:2.5. Bulk density and soil water content (SWC) were determined by oven drying to a constant mass at 105 °C. Soil organic matter (SOM) and total N (TN) were determined by dichromate digestion and Kjeldahl digestion, respectively. Total P (TP) was determined by vanadium molybdate yellow colorimetric method after $HClO_4$-H_2SO_4 digestion. The increase in H_2SO_4–extractable P after ignition (550 °C, 1 h) is assumed to be organic P (Bao, 2000). Particle size distribution was measured by the pipette method (Sheldrick and Wang, 1993). Within one week, we determined MBC, MBN, and MBP, as well as K_2SO_4-extractable C (Ext-C), N (Ext-N), and $NaHCO_3$-extractable P (Ext-P). To eliminate the effect of different water contents on fumigation and extraction, we adjusted the SWC of soil samples to 40% of water-holding capacity (WHC, determined gravimetrically after the gravitational water is lost from the sample) using deionized water first, followed by cooling the samples overnight at 4 °C to balance soil moisture.

MBC and MBN were estimated by fumigation extraction (Brookes et al., 1985; Vance et al., 1987). Briefly, soil samples (25 g dry base) were fumigated with ethanol-free chloroform for 24 h at 25 °C. After removal of the chloroform, soluble C and N were extracted from fumigated and non-fumigated samples in 100 mL of 0.5 M K_2SO_4 for 30 min on an orbital shaker. Total organic C and N in the filtered extract were determined using a TOC-N auto-analyzer (multi N/C ®3100, Jena, Germany). We converted microbial C flush (the difference in extractable C between fumigated and non-fumigated samples) to MBC using a factor of 0.45 (Vance et al., 1987) and microbial N flush to MBN using a factor of 0.54 (Brookes et al. 1985). For our current study, we used the K_2SO_4-extractable C and N from non-fumigated soil samples as a measure of Ext-C and Ext-N, respectively (Beck et al., 1997; Hamel et al., 2006). MBP was determined in triplicate using a fumigation extraction method as described by Brookes et al. (1982). The pre-treatment was in accordance with MBC and MBN. Soluble P in fumigated and non-fumigated soil samples (5 g dry base) was extracted in 100 mL of 0.5 M $NaHCO_3$ (pH 8.5) for 30 min on an orbital shaker. In addition, we added KH_2PO_4 (25 mg P/kg dry soil) to another identical sample to determine the P recovery rate for correction. We converted microbial P flush to MBP using a factor of 0.40 (Brookes et al., 1982). In the current study, we used the $NaHCO_3$-extractable P from non-fumigated soil samples as a measure of Ext-P.

2.5. Plant sampling and analysis

In our study, we did five aboveground (25th of May, 3rd of July, 14th of Aug, 24th of Sep and 16th of Oct) and four belowground (25th of May, 3rd of July, 14th of Aug, and 16th of Oct) samples in 2014 based on plant growth (see Fig. 1). We cut aboveground samples using scissors on three randomly selected areas of 1 × 1 m in each sub-plot (totally nine sample squares and three replicates for each paddock), sorted free from dead material and then enveloped the samples. Fifteen root samples of 20-cm depth were taken with a soil auger (7-cm diameter) in each sub-plot (three replicates), cleaned under running water to remove soil particles and then transferred into envelopes.

We dried all samples at 80 °C for de-enzymation immediately after sampling and then at 60 °C to determine dry matter. Then we sieved the samples over a 0.25 mm mesh and digested the sub-samples using H_2SO_4-H_2O_2. We analyzed N and P concentration in the filtered extract via the Kjeldahl method and the vanadium molybdate yellow colorimetric method, respectively (Bao, 2000).

Calculation of turnover rate and flux and plant N, P uptake

We applied the concept of McGill et al. (1986) to estimate the microbial C turnover from seasonal fluctuations in MBC to calculate the turnover rate of MBN and MBP (Eq. (1)):

$$\text{Turnover rate} = \frac{\sum \text{losses}}{\text{mean}} \tag{1}$$

With the sum of MBC (or MBN, MBP), losses ($\sum$ losses) calculated from the sum of decreases in MBC (or MBN, MBP) between two subsequent measurements, and the mean MBC (or MBN, MBP), defined as the average MBC (or MBN, MBP) of all the measurement dates, both given in mg kg^{-1} of dry soil. The turnover rate is expressed per year.

The MBC (or MBN, MBP) flux was then calculated as shown in Eq. (2):

$$\text{Flux} = \frac{\text{mean} \times \rho \times \text{D} \times \text{area}}{\text{turnover time}} \quad (2)$$

where ρ is the soil bulk density (Table 1), D is the sampling depth of 20 cm and the area is 1 ha. The turnover time is reciprocal to the turnover rate. The resulting numerator of Eq. (2) is the mean MBC (or MBN, MBP) stock (in kg ha^{-1}), and the MBC (or MBN, MBP) flux is thus given in kg ha^{-1}. We calculated the Ext-C (or Ext-N, Ext-P) stock (in kg ha^{-1}) similar to the mean MBC (or MBN, MBP) flux.

We calculated the N, P uptake based on the concept of Zhang et al. (1989) (Eqs. (3) and (4)):

$$A_{above} = B_{max-above} C_{live} \quad (3)$$

$$A_{below} = B_{max-below} T C_{aver} \quad (4)$$

$$A_{total} = A_{above} + A_{below} \quad (5)$$

where A_{above} in Eq. (3) is the annual aboveground uptake of N or P (in kg ha^{-1} $year^{-1}$), $B_{max\text{-}above}$ is the maximal aboveground biomass (kg ha^{-1} $year^{-1}$) and C_{live} is the N (or P) concentration in living aboveground biomass. In Eq. (4), A_{below} is the annual belowground uptake of N or P (in kg ha^{-1} $year^{-1}$), $B_{max\text{-}below}$ is the maximal belowground biomass (kg ha^{-1}), and T is the turnover rate of the roots ($year^{-1}$), which we derived via dividing $B_{max\text{-}below}$ by the sum of increases in root biomass. C_{aver} is the mean concentration of N (or P) of roots over a growth season. A_{total} in Eq. (5) is the annual total plant uptake.

2.6. Statistical analysis

We performed all statistical analyses with SPSS software (version 20.0; SPSS Inc., Chicago, IL) and EXCEL 2010. Due to the difficulty in establishing a proper replicated experiment for this type of work, we treated each sampling sub-plot as a replicate to carry out the analysis of variance (ANOVA). We expressed all data on a dry-weight basis and assessed the effects of management and sampling time (seasonal variation) using a repeated-measures ANOVA. We tested all variables for normality of the distribution before analysis. In all cases, we considered $P < 0.05$ as significant. Stepwise multiple regression analyses were used in order to examine the relationships among several environmental factors and short term changes of MBC, MBN and MBP during each sampling interval. For MBC, the factors included change in air temperature (DLTTEMPERATURE), rainfall (DLTRAINFALL), soil water content (DLTSWC), Ext-C (DLTExt-C), Ext-N (DLTExt-N), and Ext-P (DLTExt-P). Apart from these factors, change in MBC (DLTMBC) and MBP (DLTMBP) were also included for MBN. As for MBP, change in MBN (DLTMBN) was involved instead of DLTMBP. If the F test was significant at $P < 0.05$, we calculated the least significant difference (LSD) with an alpha of 0.05 to compare the index related to turnover and plant nutrient uptake in different treatments.

3. Results

3.1. Seasonal climatic variations

The daily average precipitation and air temperature during the experimental period are shown in Fig. 1. Generally, during the experimental period the air temperature gradually rose, reached a peak in July and August and then started to decline. There were many fluctuations observed over a year and across the two years.

The precipitation in 2014 was 255 mm with a tremendously uneven distribution (Fig. 1). As a result, SWC varied across season from 0.03 to 0.14 g g^{-1} (coefficient of variation (CV) 30.3%–36.3%). In E83 and E96, SWC were significantly higher than in FG except for dates 10th June and 22nd July 2014 (both $P < 0.001$) (Fig. 2).

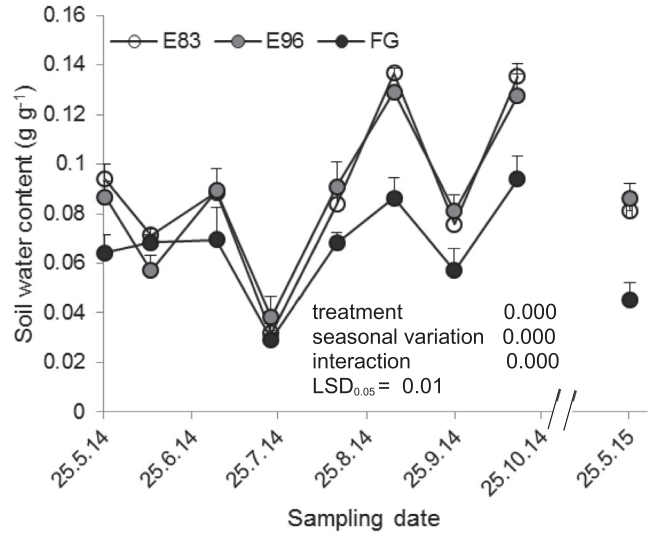

Fig. 2. Soil water content in grassland enclosed from grazing since 1983 (E83) or since 1996 (E96) or with continuously free grazing (FG) at each sampling date (May 2014 to May 2015). Means and standard deviations of the three field replicates. Significance levels of two-factorial ANOVA with main factors effect and their interaction shown in the figure. The LSD refers to the interaction between treatment and seasonal variation.

3.2. Seasonal variations in soil microbial biomass and nutrients

Overall, MBC, MBN and MBP differed significantly along the sampling timeframe. Each of the microbial pools presented similar fluctuation patterns among the three treatments but at different levels. MBC and MBN were significantly higher in E83 and E96 than in FG. No difference in MBP among the three experimental sites was detected (Fig. 3). The overall MBC, MBN, and MBP averaged 216 μg g^{-1}, 32 μg g^{-1}, 24 μg g^{-1}, accounting for in average 1.4%, 2.2%, and 7.4 of the total soil organic C (SOC), TN, and TP, respectively (Fig. 3, Table 1).

Season also affected soil available nutrients (Fig. 4). In E83, the averages for Ext-C, Ext-N, and Ext-P were 37.6 μg C g^{-1}, 8.3 μg N g^{-1}, and 7.8 μg P g^{-1}, respectively. In E96, they were 33.3 μg C g^{-1}, 7.9 μg N g^{-1}, and 10.1 μg P g^{-1}, respectively. In FG, they were 30.9 μg C g^{-1}, 6.7 μg N g^{-1}, and 9.6 μg P g^{-1}, respectively (Fig. 4). Compared with FG, the soil annual Ext-C ($P < 0.01$) and Ext-N ($P < 0.01$) significantly increased in E83, while we found no significant impact of enclosure on Ext-P (Fig. 4).

The changes of MB C:N, C:P, and N:P ratios are listed in Table 2. The MB C:N ratio during the season was rather constant except for 16th Oct 2014 and no significant differences were observed among treatments. In contrast, MB C:P ratios in E83 and E96 and MB N:P ratio in E83 were higher than those in FG. The ratios of MB C:P and MB N:P tend to decrease in the early stages of the season (between 25th May 2014 and the subsequent time points).

3.3. Factors controlling soil microbial biomass dynamics

Stepwise multiple linear regression analyses were carried out to study factors controlling soil microbial biomass dynamics (Table 3). Across all treatments, major controls on MBC variation were the changes in temperature, Ext-N, and Ext-C, which accounted for 47%, 12%, and 10%, respectively. The change in MBC, as a factor itself, controlled the variability of MBN (accounting for 87%) and MBP (accounting for 21%). In addition, the change in soil water content and in temperature also controlled some of the MBN variation (r^2 were 0.086 and 0.01, respectively).

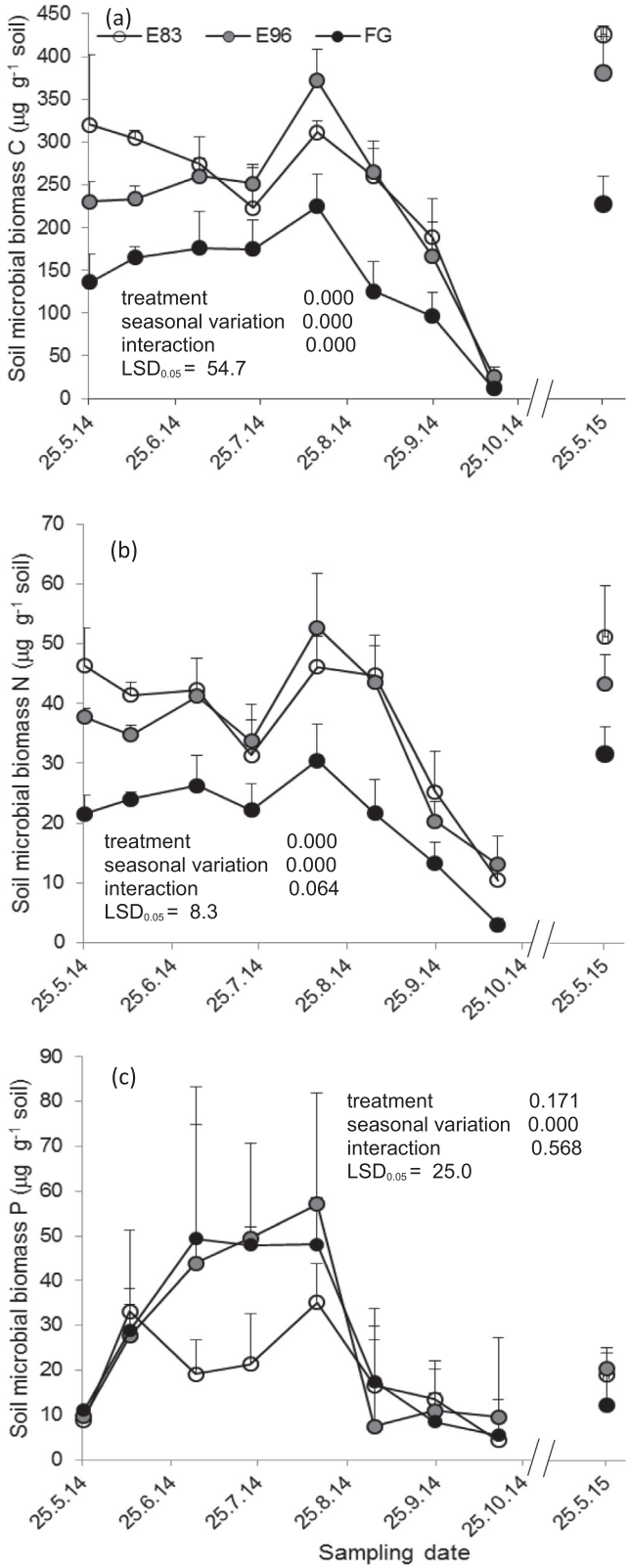

Fig. 3. Seasonal fluctuations of soil microbial biomass C (a), N (b), and P (c) for E83, E96 and FG (see Fig. 2 for the descriptions) during the experimental period (May 2014 to May 2015). Means and standard deviations of the three field replicates. Significance levels of two-factorial ANOVA with main factors effect and their interaction shown in the figure. The LSD refers to the interaction between treatment and seasonal variation.

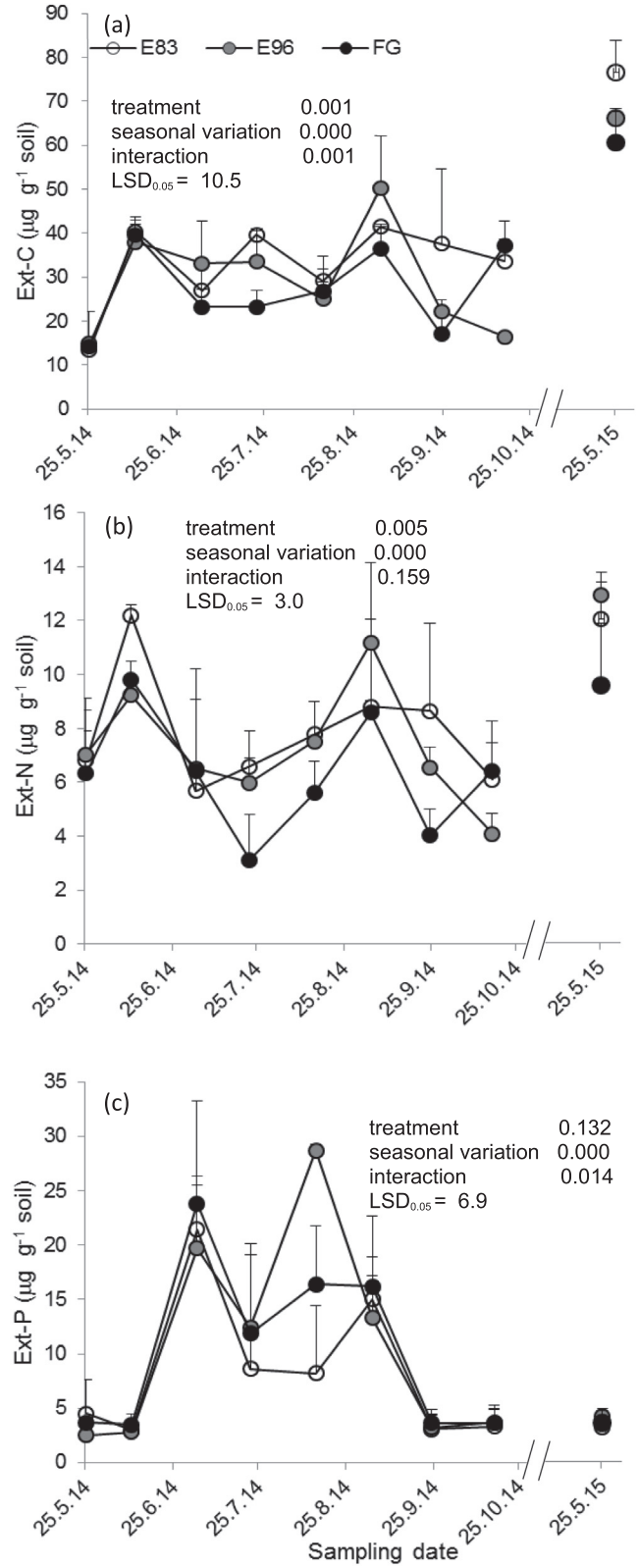

Fig. 4. Seasonal fluctuations of soil extractable C (with 0.5 M K_2SO_4, Ext-C) (a), N (with 0. 5 M K_2SO_4, Ext-N) (b), and P (with 0.5 M $NaHCO_3$, Ext-P) (c) for E83, E96 and FG (see Fig. 2 for the descriptions) during the experimental period (May 2014 to May 2015). Means and standard deviations of the three field replicates. Significance levels of two-factorial ANOVA with main factors effect and their interaction shown in the figure. The LSD refers to the interaction between treatment and seasonal variation.

Table 2
Seasonal fluctuations and summary of average of soil microbial C:N ratio, N:P ratio, and C:P ratio for E83, E96 and FG[a] during the experimental period (May 2014 to May 2015). The results of the repeated-measures ANOVA for the effects of treatment (T) and seasonal variation (S) are given in the table. Note that the effects of interaction on the ratios in all treatments are not significant and thus are not listed.

Microbial ratio	Treatment	Sampling date (day/month/year)									Average	Effect	
		25/5/2014	10/6/2014	3/7/2014	22/7/2014	14/8/2014	3/9/2014	24/9/2014	16/10/2014	25/5/2015		T	S
C:N	E83	6.8 ± 0.8	7.0 ± 0.4	6.5 ± 0.2	7.0 ± 0.4	7.3 ± 0.9	5.8 ± 0.3	7.5 ± 0.4	1.3 ± 0.1	8.4 ± 1.1	6.4	P = 0.526	P < 0.001
	E96	6.1 ± 0.5	7.4 ± 0.1	6.3 ± 0.4	7.5 ± 0.4	7.0 ± 1.0	6.1 ± 0.4	8.1 ± 1.0	2.6 ± 0.7	8.8 ± 0.1	6.7		
	FG	6.3 ± 0.7	6.9 ± 0.6	6.7 ± 0.5	7.9 ± 0.2	7.5 ± 1.9	5.9 ± 1.2	7.2 ± 0.4	4.6 ± 2.0	7.3 ± 1.2	6.7		
C:P	E83	38.1 ± 6.9	9.2 ± 0.5	15.4 ± 4.7	13.4 ± 8.5	9.4 ± 3.0	11.1 ± 2.7	19.5 ± 13.4	2.0 ± 0.4	23.3 ± 6.3	15.6	P = 0.050	P = 0.004
	E96	23.8 ± 2.4	9.0 ± 2.4	9.9 ± 9.3	5.7 ± 3.7	6.5 ± 0.5	4.5 ± 17.6	33.9 ± 35.0	6.4 ± 14.8	19.4 ± 5.9	13.3		
	FG	12.3 ± 12.3	8.4 ± 3.0	5.8 ± 4.9	3.7 ± 10.6	6.2 ± 2.5	9.2 ± 2.2	14.8 ± 14.3	1.7 ± 1.3	22.5 ± 11.7	9.4		
N:P	E83	5.7 ± 1.4	1.3 ± 0.1	2.4 ± 0.8	1.9 ± 1.1	1.3 ± 0.2	2.0 ± 0.5	2.5 ± 1.6	1.6 ± 0.2	2.8 ± 0.7	2.4	P = 0.042	P = 0.003
	E96	3.9 ± 0.2	1.4 ± 0.4	1.5 ± 1.4	0.8 ± 0.6	0.9 ± 0.1	0.6 ± 2.9	1.4 ± 0.3	1.8 ± 4.0	2.2 ± 0.7	1.6		
	FG	2.0 ± 0.3	1.3 ± 0.9	0.9 ± 0.9	0.5 ± 0.1	0.8 ± 0.3	1.5 ± 0.8	2.0 ± 1.0	0.3 ± 0.1	3.0 ± 1.1	1.4		

Means ± standard deviation.
[a] See Table 1 for the descriptions of E83, E96, and FG.

Table 3
Increase in r^2 for net changes of microbial biomass C (MBC), N (MBN), and P (MBP) in all treatments[a] using stepwise multiple linear regression analyses for eight intervals between 2014 and 2015.

Parameter	Factor/r^2		
MBC	DLTTEMPERATURE	DLTExt-N	DLTExt-C
	0.469	0.116	0.095
MBN	DLTMBC	DLTSWC	DLTTEMPERATURE
	0.874	0.086	0.01
MBP	DLTMBC		
	0.214		

[a] See Table 1 for the descriptions of the three treatments.

3.4. Plant biomass and N, P uptakes

Both above- and belowground biomass were found to be significantly ($P < 0.05$) larger in E83 and E96 than those in FG (Table 4). The largest aboveground biomass observed in E83, E96, and FG was in September (0.9 t ha^{-1}, 0.9 t ha^{-1} and 0.5 t ha^{-1}, respectively), while the highest belowground biomass were 10.3 t ha^{-1}, 10.9 t ha^{-1} and 8.4 t ha^{-1} being observed in July, respectively (Table 4). Similarly, the N, P uptakes into both above- and belowground biomass were found higher in E83 and E96 than those in FG. As a result, the annual total (above- and belowground) N, P uptakes were higher in E83 (63.4 kg ha^{-1} year^{-1} and 3.4 kg ha^{-1} year^{-1}) and E96 (74.5 kg ha^{-1} year^{-1} and 4.5 kg ha^{-1} year^{-1}) than those in FG (56.1 kg ha^{-1} year^{-1} and 2.7 kg ha^{-1} year^{-1}) and were in the order E96 > E83 > FG (Table 4).

3.5. MBC, MBN, MBP turnover rates and fluxes

The turnover times of MBC, MBN and MBP were 214–235, 244–254 and 105–127 days, respectively. The turnover time of MBP was only 45–58% that of MBC and 41–52% that of MBN (Table 5). Enclosure had no significant impact on the turnover rate and time (Table 5). MBC fluxes were significantly ($P < 0.05$) higher in enclosure treatments than in FG. MBN fluxes in enclosure tended to increase, while MBP flux in E83 tended to decrease although not significantly. The mean stocks of MBC, MBN and MBP in the three treatments were similar to the results of fluxes. In all three treatments, no significant difference of the mean stocks of soil Ext-C and Ext-N was observed. However, the mean Ext-P stock in E83 significantly decreased (Table 5). MBN fluxes and mean stocks were markedly higher than the total plant N uptake, but the mean Ext-N stocks were distinctly lower than the total plant N uptake throughout all treatments (Tables 4 and 5). In contrast, the MBP fluxes, mean stocks of MBP and Ext-P were much higher than total plant P uptake throughout all treatments (Tables 4 and 5).

4. Discussion

4.1. Soil microbial pools and seasonal dynamics

In the present study, the averaged MBC, MBN and MBP (216 μg g^{-1}, 32 μg g^{-1} and 24 μg g^{-1}, respectively) were generally low compared with other natural systems (e.g. Gallardo et al., 2000; Joergensen et al., 1995; Wardle, 1992). The SOC, TN, and TP pools in this ecosystem could explain these values as the concentrations of C, N, and P in soil microbial biomass accounted for the corresponding pools of 1.4%, 2.2%, and 7.4%, respectively, which were similar to the mean estimates of 1.2%, 2.6%, and 8.0% globally (Xu et al., 2013). These constant proportions could also explain the consistently higher MBC and MBN pools in E83 and E96 than in FG across seasons (Fig. 3a and b; Table 1). In terms of MBP, no obvious difference was observed among the three experimental sites due to the small variation of total P pools (Fig. 3c; Table 1). Thus, our hypothesis that the sizes of soil microbial pools in

Table 4
Above- and belowground plant biomass and N, P uptake at each sampling date, as well as annual above- and belowground, and total N, P uptake in 2014 for E83, E96 and FG.[a]

		Treatment	Sampling date (day/month/year)					Annual uptake[b] (kg ha^{-1} year^{-1})
			25/5/2014	3/7/2014	14/8/2014	24/9/2014	16/10/2014	
Aboveground	Biomass (t ha^{-1})	E83	0.3 ± 0.1a	0.8 ± 0.1a	0.9 ± 0.0a	0.9 ± 0.1a	0.1 ± 0.1a	na
		E96	0.3 ± 0.0a	0.9 ± 0.1a	0.7 ± 0.1b	0.9 ± 0.2a	0.2 ± 0.1a	na
		FG	0.1 ± 0.0b	0.2 ± 0.0b	0.4 ± 0.1c	0.5 ± 0.1b	0.02 ± 0.01b	na
	N (kg ha^{-1})	E83	5.8 ± 0.5a	14.4 ± 1.1b	19.6 ± 5.6a	7.2 ± 0.5a	0.9 ± 0.1b	19.6 ± 5.6a
		E96	6.0 ± 0.6a	17.9 ± 1.9a	15.9 ± 4.3ab	7.8 ± 1.1a	2.1 ± 0.2a	17.9 ± 1.9a
		FG	1.2 ± 0.1b	4.8 ± 0.2c	10.2 ± 0.3b	4.1 ± 0.1b	0.3 ± 0.0c	10.2 ± 0.3b
	P (kg ha^{-1})	E83	0.5 ± 0.0b	1.2 ± 0.1b	1.1 ± 0.1a	0.5 ± 0.1b	0.1 ± 0.0b	1.2 ± 0.1b
		E96	0.6 ± 0.0a	1.5 ± 0.1a	0.8 ± 0.1b	0.7 ± 0.1a	0.2 ± 0.0a	1.5 ± 0.1a
		FG	0.1 ± 0.0c	0.3 ± 0.0c	0.4 ± 0.0c	0.3 ± 0.0c	0.02 ± 0.00c	0.4 ± 0.0c
Belowground	Biomass (t ha^{-1})	E83	8.5 ± 1.7a	10.3 ± 1.9a	7.6 ± 1.1a	/	7.1 ± 2.3a	na
		E96	8.3 ± 0.9a	10.9 ± 1.9a	7.5 ± 1.0a	/	8.2 ± 2.1a	na
		FG	5.2 ± 0.2b	8.4 ± 2.4a	4.8 ± 0.2b	/	3.1 ± 1.0b	na
	N (kg ha^{-1})	E83	149.9 ± 13.5a	160.9 ± 3.3a	129.9 ± 8.1a	/	107.2 ± 8.7a	43.8 ± 1.4b
		E96	131.3 ± 9.5a	169.0 ± 8.0a	130.8 ± 6.2a	/	107.5 ± 3.6a	56.6 ± 1.7a
		FG	79.0 ± 11.4b	123.0 ± 3.4b	71.0 ± 7.3b	/	39.6 ± 7.9b	45.9 ± 2.7b
	P (kg ha^{-1})	E83	7.6 ± 0.6a	8.0 ± 0.4a	6.4 ± 0.5a	/	6.0 ± 0.2a	2.2 ± 0.1b
		E96	7.0 ± 0.3a	7.8 ± 0.7a	6.3 ± 0.2a	/	7.1 ± 0.8a	3.0 ± 0.2a
		FG	3.7 ± 0.1b	5.7 ± 0.9b	3.7 ± 0.0b	/	2.4 ± 0.5b	2.3 ± 0.2b
Annual total uptake[c]	N (kg ha^{-1}year^{-1})	E83	na	na	na	na	na	63.4 ± 7.0b
		E96	na	na	na	na	na	74.5 ± 3.7a
		FG	na	na	na	na	na	56.1 ± 3.0b
	P (kg ha^{-1} year^{-1})	E83	na	na	na	na	na	3.4 ± 0.2b
		E96	na	na	na	na	na	4.5 ± 0.3a
		FG	na	na	na	na	na	2.7 ± 0.2c

Means ± standard deviations. Different letters along the column indicate significant differences between mean values of each parameter among different management regimes at $P < 0.05$.
/ means not measured.
na means not applicable.
[a] See Table 1 for the descriptions of E83, E96, and FG.
[b] Calculated according to Eqs. (3) and (4).
[c] Calculated according to Eq. (5).

Table 5
Calculated microbial biomass C (MBC), N (MBN), and P (MBP) turnover and fluxes, as well as stocks of MBC, MBN, MBP and Ext-C, Ext-N, Ext-P in the top 20 cm of the soil in the three management regimes E83, E96 and FG.[a]

		E83	E96	FG	$LSD_{0.05}$
Σof losses (mg kg^{-1})	MBC	422 ± 41	380 ± 45	253 ± 7	70
	MBN	54.9 ± 6.9	52.1 ± 9.0	33.3 ± 8.7	16.5
	MBP	56.6 ± 12.1	93.5 ± 17.7	81.1 ± 30.9	43.4
Turnover rate (year^{-1})[b]	MBC	1.6 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	0.3
	MBN	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.3	0.5
	MBP	3.0 ± 0.7	3.6 ± 0.6	3.1 ± 0.4	1.2
Turnover time (days)	MBC	224 ± 23	235 ± 28	214 ± 19	47
	MBN	253 ± 32	254 ± 36	244 ± 47	78
	MBP	127 ± 35	105 ± 21	118 ± 13	49
Flux (kg ha^{-1} year^{-1})[c]	MBC	1011 ± 98	1031 ± 121	762 ± 21	181
	MBN	132 ± 17	141 ± 24	101 ± 26	46
	MBP	136 ± 29	253 ± 48	245 ± 93	126
Mean stock (kg ha^{-1})	MBC	617 ± 39	659 ± 8	447 ± 33	60
	MBN	90.3 ± 5.4	96.6 ± 2.0	65.0 ± 5.5	9.2
	MBP	45.6 ± 1.9	71.2 ± 2.9	76.8 ± 20.4	23.8
	Ext-C	90.1 ± 4.7	90.2 ± 8.3	95.5 ± 9.6	15.6
	Ext-N	19.9 ± 0.9	21.4 ± 2.9	20.0 ± 1.4	3.8
	Ext-P	19.2 ± 3.6	27.3 ± 3.5	28.9 ± 1.8	5.9

Means ± standard deviations. Least significant differences (LSD) calculated for $\alpha = 0.05$, with Holm adjusted P values.
[a] See Table 1 for the descriptions of E83, E96, and FG.
[b] Calculated according to Eq. (1).
[c] Calculated according to Eq. (2).

enclosure treatments are larger during growth season is supported in MBC and MBN, but not in MBP.

We found an average SOC:TN:TP molar ratio of 129:10:1 in these grassland soils, which was lower than previously reported in a global analysis (287:17:1). As a result, the C:N:P ratio recorded in soil microbial biomass (25:3:1) in this ecosystem was also lower (around 2 times) than global analysis (46:6:1) (Xu et al., 2013). Our results partly agree that microbial biomass stoichiometry (microbial C:N ratio) is well constrained (Cleveland and Liptzin, 2007; Xu et al., 2013), but with low levels of microbial C:P and N:P ratios. This may suggest that N is more limiting than P for microorganisms in this grassland ecosystem. Furthermore, the two ratios were even lower in the grazed grassland (Table 2), indicating a tendency to N limitation of soil microbes by grazing. This could be explained by the higher N:P ratio of aboveground uptake in FG (Table 4), which would be typically removed out of the system by animals whereas the plant residue in enclosure treatments would eventually return to the soils. Consequently, the long-term loss of 'more' N and 'less' P resulted in the lower soil N pool and microbial N:P ratio in grazing area.

Soil MBC, MBN and MBP showed distinct seasonal changes (Fig. 3). Interestingly, rapid declines in MBC, MBN and MBP from 14th of Aug to 16th of Oct 2014 and recoveries by May 2015 were detected. The results were not in accordance with Patra et al. (1990) who reported near constant seasonal MBC, MBN and MBP for a UK grassland soil. However, the authors detected a sharp decline in released CO_2 from August to December, indicating a decreasing microbial activity in winter (Patra et al., 1990). Based on the stepwise multiple linear regression analyses, 68% of the MBC variability could be accounted for by the combined effects of the changes of air temperature, Ext-N, and Ext-C (Table 3). The change in MBC largely influenced MBN dynamics (accounted for 87% of the variability), which is also evidenced by the constant MB C:N ratio across the season (Tables 2 and 3). Seasonal changes in soil moisture and temperature could directly affect microbial biomass and may also operate indirectly through modulating substrate availability through plant phenology (Carter and Rennie, 1984; Rinnan et al., 2008). Our results confirmed the effect of temperature but not SWC on soil microbial biomass. This could be explained by the small variations of SWC (Fig. 2), or microbial adaptation to the fluctuations in soil moisture since severe dry-wet cycles frequently occurred in soils in semiarid grassland (Fierer and Schimel, 2002; Sugihara et al., 2015). Available nutrient pools, which control microbial processes such as mineralization and immobilization (Schmidt et al., 1999), changed over the seasons (Fig. 4). The increase of plant N uptake during rapid growth season accompanied by the decreases of Ext-N (Fig. 4b, Table 4) and MBN (Fig. 3b) indicates N competition between plants and soil microbes and may imply N deficiency in the studied area. In contrast, the plant growth and P uptake did not affect the increase of MBP (Fig. 3c; Table 4), which was accompanied by the increase of Ext-P, indicating no P deficiency existing at current N status.

4.2. Turnover and contributions of microbial biomass to plant nutrient uptake

The turnover rates of MBC, MBN and MBP were similar in the three treatments, which did not support our hypothesis that enclosure (relatively rich nutrient condition compared to grazing area) would increase the turnover rate of soil microbial biomass. This was evidenced by the similar trends of microbial pools among the three treatments across seasons (Fig. 3), suggesting that climatic conditions, and not grassland management affected microbial turnover rate in this area. This agrees with Wu and Xiao (2004) who reported soil texture and temperature as the main influencing factors for soil microbial turnover rate, while land-use patterns had only a minor effect. Benesch et al. (2015) considered soil humidity to be the main influencing factor for microbial turnover of litter-derived C in forest ecosystem. Our three adjacent sites had the same soil texture and climatic conditions, which might result in similar microbial turnover.

Wu and Xiao (2004) estimated the turnover rate of MBC in the Rothamsted Experimental Station as 0.24–1.0 $year^{-1}$. Von Lützow and Ottow (1994) calculated turnover rates of MBC and MBN in the arable soils of Germany as 0.3–1.2 $year^{-1}$ and 0.9–1.1 $year^{-1}$, respectively. The turnover rates of MBC and MBN estimated in the present study (1.6 $year^{-1}$ and 1.5 $year^{-1}$) were slightly faster than those reported previously. In terms of MBP, the turnover (3.0–3.6 $year^{-1}$) was also faster than that reported for a permanent grassland soil in Switzerland (1.2–1.6 $year^{-1}$) (Liebisch et al., 2014). Besides, the turnover of MBP was also faster than that of MBN (Table 5). This could be due to P entering a part of the biomass that is continuously degraded and resynthesized within living cell, and more easily degradable after cells death (Kouno et al., 2002). Furthermore, the relative N limitation for soil microbes would slow down its turnover by increased efficiency of internal element retaining and recycling (Spohn and Widdig, 2017). We suggest the size of microbial pools would be the key factor controlling the turnover fluxes due to similar turnover rates among treatments (based on Eqs. (1) and (2)). For example, Hong et al. (1997) reported that the fluxes of MBC in a German grassland soil with high SOC was 8539 kg ha^{-1} $year^{-1}$, which was much higher than that calculated in the present study (Table 5). Liebisch et al. (2014) reported higher MBP turnover fluxes in the P addition treatments with higher MBP pools than in the treatment without P addition, while the turnover rates in all treatments remained the same.

Both above- and belowground biomass were lower in FG than those in enclosure treatments (Table 4), indicating nutrient deficiency in FG. To be more specific, Ext-N stock was lower than MBN stock that was 43%, 30% and 16% higher than the annual N uptake in E83, E96 and FG, respectively (Tables 4 and 5). This indicates that the N deficiency in soils was better counteracted by the N pool in microbial biomass in E83 and E96 than in FG in this region. In contrast, Ext-P stock was 6–11 times greater than the annual P uptake in each experimental site (Tables 4 and 5), indicating P saturation under current N status. Our results agree with the observation of Gong et al. (2011) in the same ecosystem that P limitation came after N limitation. However, we cannot deduce the effects of soil microbial nutrient pools as direct sources on plant nutrient uptake. Further studies are necessary to better understand nutrient fluxes in soil-microbe-plant systems in this region.

5. Conclusions

In the studied semiarid grassland, while the size of soil microbial biomass pool was affected by pasture management, the short term biomass dynamics were controlled by season. Our results confirmed that microbial C:N ratio is well constrained (homeostatic), but showed low level of microbial C:P and N:P ratios compared with global analysis, suggesting N would be more limiting than P for microorganisms in this ecosystem. The deduction was evidenced by the observed competition for N but not P between microbes and plants during the growth season.

Grassland management affects nutrient fluxes due to microbial turnover mainly through its effect on pool sizes since the turnover rates were similar in all management regimes. Double faster turnover of MBP than MBN again supported N deficiency for soil microbes. Lower mean stock in Ext-N but similar in MBN compared with total plant N uptake suggests N deficiency in the studied ecosystem. In contrast, much higher stocks in both Ext-P and MBP compared with total plant P uptake indicate no P deficiency under current N status.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.apsoil.2018.04.008.

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