Biogeosciences Discuss., doi:10.5194/bg-2016-483, 2016
Manuscript under review for journal Biogeosciences
Published: 10 November 2016

Biogeosciences Discussions
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Alteration of carbon, nitrogen, and phosphorus stoichiometry and their related enzymes as affected by increased soil coarseness

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Biogeosciences Discuss., doi:10.5194/bg-2016-483, 2016
Manuscript under review for journal Biogeosciences
Published: 10 November 2016

Abstract

Soil coarseness decreases ecosystem productivity, ecosystem carbon and nitrogen stocks, and soil nutrient contents in sandy grasslands. To gain insight into changes in soil carbon and nitrogen pools, microbial biomass, and enzyme activities in response to soil coarseness, a field experiment of sand addition was conducted to coarsen soil with different intensities: 0% sand addition, 10%, 30%, 50%, and 70%. Soil organic carbon and total nitrogen decreased with the intensification of soil coarseness across three depths (0-10 cm, 10-20 cm, and 20-40 cm) by up to 43.9% and 53.7%, respectively. At 0-10 cm, soil microbial biomass carbon (MBC) and nitrogen (MBN) declined with soil coarseness by up to 44.1% and 51.9%, respectively, while microbial biomass phosphorus (MBP) increased by as much as 73.9%. Soil coarseness significantly decreased the activities of β-glucosidase, N-acetyl-glucosaminidase, and acid phosphomonoesterase by 20.2%-57.5%, 24.5%-53.0%, and 22.2%-88.7%, respectively. Soil coarseness enhanced microbial C and N limitation relative to P, indicated by the ratios of β-glucosidase and N-acetyl-glucosaminidase to acid phosphomonoesterase (and MBC:MBP and MBN:MBP ratios). As compared to laboratory measurement, values of soil parameters from theoretical sand dilution was significantly lower for soil organic carbon, total nitrogen, dissolved organic carbon, total dissolved nitrogen, available phosphorus, MBC, MBN, and MBP. Phosphorus immobilization in microbial biomass might aggravate plant P limitation in nutrient-poor grassland ecosystems as affected by soil coarseness. We conclude that microbial C:N:P and enzyme activities might be good indicators for nutrient limitation

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of microorganisms and plants.

Key words sandy grassland, grassland degradation, microbial biomass, microbial nutrient limitation, soil carbon stocks

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1 Introduction

Soil coarseness is one of the principle constrains to terrestrial net primary productivity (NPP), ecosystem health, and regional economy (Lal, 2014; Lü et al., 2016). Desertification and wind erosion processes are main contributors of soil coarseness in arid and semi-arid grasslands (Su et al., 2004; Lü et al., 2016). By the 1990s, more than 74% of world total dryland area, 100 countries, and 0.85 billion people have been influenced by desertification and soil coarseness (Chang et al., 2015). Abundant evidence has confirmed that desertification and soil coarseness decreases NPP (Peters et al., 2012), soil organic carbon (SOC) storage in both soil and plant components (Zhou et al., 2008), and soil nutrient retention (Delgado-Baquerizo et al., 2013). In response to desertification and soil coarseness, the decrease of NPP would pose threat to world food security (Zhao et al., 2006); loss of SOC would enhance carbon-climate feedback (Lal, 2014); and decline in soil nutrient retention would cause soil deterioration and loss of soil structure (Su et al., 2004). Therefore, it is important to characterize impacts of soil coarseness on ecosystem processes in order to understand the mechanisms that cause desertification.

Microbes play a particularly important role in regulating plant nutrient availability in nutrient-poor environments (van der Heijden et al., 2008). Microbial biomass is less than 4% of soil organic C, but it makes substantial contributions to stable soil C formation and major nutrient supply (Brookes, 2001; Liang and Balser, 2011). For instance, microbial biomass phosphorus (MBP) has been regarded as a central feature in P cycling and plays an essential role in soil organic P mineralization

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(Richardson and Simpson, 2011). Soil nutrient supply is predominately controlled by microbial decomposition of soil organic matter (SOM) (excepting P which can be supplied by rock weathering) (Balota et al., 2014) and this process mainly relies on extracellular enzymes secreted by microorganisms and plants (Tabatabai, 1994; Wang et al., 2015). However, C and nutrient availabilities in soil environments can constrain this kind of essential microbial function (Cleveland et al., 2002) and be reflected by enzymatic stoichiometry and kinetics (Sinsabaugh et al., 2008, 2014; Wang et al., 2015). For instance, microbial P limitation decreased soil microbial respiration and SOM decomposition, which could profoundly influence C cycling in tropical forests (Cleveland et al., 2002). Lower ratios of soil β-glucosidase (BG) to acid phosphatase (PME) and N-acetyl-glucosaminidase (NAG) to PME illustrated greater microbial P demand relative to C and N, respectively (Waring et al., 2014; Wang et al., 2015). Though a spate of studies have investigated desertification and soil coarseness effects on plant productivity (Zhao et al., 2006), composition of soil particle sizes (Su et al., 2004; Zhao et al., 2006), soil C and N dynamics (Zhou et al., 2008), and soil nutrient availabilities to plants (Zhao et al., 2006; Li et al., 2013), researches concerning microbial biomass C, N and P contents, soil enzyme activities, and microbial nutrient limitations are still rarely seen. Under soil coarseness conditions, stoichiometry of soil microbial biomass C:N:P and extracellular enzymes remain largely unknown (Cleveland and Liptzin, 2007; Sinsabaugh et al., 2008).

The Horqin Sandy Grassland is one of the main parts of Inner Mongolia grassland belonging to Eurasian steppe. At the southeastern edge of Horqin Sandy Grassland,

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Zhangwu County used to be productive steppe grassland until 1950s when overgrazing and over-cultivation happened to support rapidly growing human population (Li et al., 2004). After decades of over-utilization, the natural grassland has turned into an agro-pastoral zone and has undergone severe desertification and ecosystem retrogression (Yu et al., 2008). Soil coarseness is common in this area resulting from significant decreases in plant coverage and high annual wind speed (varying from 3.4 to 4.1 m s^{-1}) with frequent occurrence of gales (wind speed > 20 m s^{-1}) (Lü et al., 2016). In previous work we showed that soil pH, soil fine particles (< 250 μm), soil exchangeable Ca and Mg, and soil available Fe was significantly decreased by soil coarseness (Lü et al., 2016). In this study, we hypothesized that 1) soil coarseness would decrease both soil C and N contents as well as their stocks across soil depths; 2) soil coarseness would decrease microbial C, N, and P as well as the activities of C-, N-, and P-cycling enzymes because of the significant decrease in SOM; 3) soil coarseness would increase soil microbial C and N limitation relative to P as P could be supplied through abiotic processes.

2 Materials and methods

2.1 Study site and experimental design

The field experiment was located in Zhanggutai Town (42°43′N, 122°22′E, elevation 226.5 m a.s.l.) at the southeast of Horqin Sandy Land of northern China. The mean annual temperature is 6.3 ℃ and the mean annual precipitation is 450 mm. The soil is an Aeolic Eutric Arenosol in the FAO classification (IUSS Working Group

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WRB, 2014).

In 2011, treatments were established on 4 m × 4 m plots arranged in a complete randomized design with five treatments and six replicates. Original plants were removed before preparing the soil. To simulated different soil coarseness degrees, soils from three soil depths (0-20 cm, 20-40 cm, and 40-60 cm) were dug out and mixed with 2 mm-sieved river sand in different mass proportions evenly and then refilled back. The mixing proportions are 0 (C0), 10% (C10), 30% (C30), 50% (C50), and 70% (C70) of soil mass. The river sand contains 1.29±0.04 g kg^{-1} C and 0.15±0.03 g kg^{-1} N with a pH of 7.5±0.2. In August 2012, soils of 0-5 cm depth were taken out from all plots and sterilized at 105 ℃ for 3h to deactivate the seeds and prevent plant growth. The 0-5 cm soils were filled back and treatment plots were equilibrated for 1 year until July 2013. After 1-year equilibration, native plants were transplanted from local grassland according to native community composition. The purpose of deactivation of seeds and transplantation were to build the same plant community as native grassland. Since 2014, a permanent quadrat of 1 m × 1 m was set up within each plot to investigate plant community composition at August.

2.2 Soil sampling and chemical analysis

In October 2015, soil samples (0-10 cm, 10-20 cm, and 20-40 cm) were taken by compositing three randomly placed soil cores within each plot. Fresh samples were passed through a 2 mm sieve, sealed in plastic bags, and stored at 4 ℃ until further processing.

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For three soil layers, the contents of SOC and TN were determined on air-dried and ground soils using an elemental analyzer (Vario MACRO Cube, Elementar, Germany). Sulfanilamide (C = 41.81%, N = 16.25%) was used as the internal standard. The SOC or TN stocks of three soil layers were calculated by multiplying SOC or TN contents with soil bulk density. The soil dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were extracted from 15 g fresh soils by 60 ml of 0.5 M K_2SO_4 and filtered through 0.45 μm acetate filter paper after shaking at 120 rpm for 1 h (Wang et al. 2015). The contents of DOC and TDN in filtrate were determined by a TOC analyzer (Multi N/C 3100, Analytikjena, Germany).

Soil pH was measured in a 1:2.5 (w/v) soil-to-water slurry using a PHS-3G digital pH meter (Precision and Scientific Crop., Shanghai, China). Soil particle size distribution was measured according to Zhao et al. (2006) by the pipette method. Soil fine particle in the size of < 250 μm was used in this study and calculated by the sum of fine sand, silt and clay. Soil exchangeable Ca and Mg were extracted by 1 M CH_3COONH_4 solution (Ochoa-Hueso et al. 2014). Available Fe was extracted by diethylenetriaminepentaacetic acid (DTPA) (Lindsay and Norvell 1978). Soil exchangeable Ca and Mg contents and Fe availability were analyzed by atomic absorption spectrometer (AAS, Shimazu, Japan).

2.3 Microbial biomass and enzyme activities

Microbial biomass C (MBC) and N (MBN) were measured using the fumigation-extraction method (Brooks et al., 1985). Soil subsamples of 15 g were

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fumigated with chloroform ($CHCl_3$) at 25 ℃ for 24 h and non-fumigated subsamples were kept at the same conditions. After fumigation, both fumigated and non-fumigated samples were extracted with 0.5 M K_2SO_4 in a 1:4 (w/v) soil-to-extractant ratio and shaken at 150 rpm for 1 h. After filtration, the soil extracts were analyzed by a TOC analyzer (Multi N/C 3100, Analytikjena, Germany) for extractable C and N contents. Microbial biomass P was determined by extracting fumigated (also by $CHCl_3$) and non-fumigated soils with 0.5 M $NaHCO_3$ (pH 8.5) (Brookes et al., 1982). Briefly, 15 g of both fumigated and non-fumigated soil samples were mixed with 60 ml 0.5 M $NaHCO_3$ and shaken at 150 rpm for 1 h. After filtration, the extractable P content in filtrate was determined by the molybdenum blue colorimetric method (Murphy and Riley, 1962). The measured P conctent in unfumigated soil samples was soil Olsen-P (Wang et al., 2016). To correct for incomplete extraction, we used efficiency factor of 0.45, 0.54, and 0.40 to calculate the actual contents of MBC, MBN and MBP, respectively (Dijkstra et al., 2012).

Enzyme assays for β-glucosidase (BG), N-acetyl-glucosaminidase (NAG) and acid phosphomonoesterase (PME) were performed on frozen and fresh sieved soil samples. For BG activity, 1.0 g of soil sample was mixed with a pH 6.0 modified universal buffer (0.1 M trihydroxymethyl aminomethane + 0.067 M citric acid monohydrate compound + 0.1 M boric acid). The *p*-nitrophenyl-β-D-glucopyranoside (0.05 M) was added as indicator substrate to the mixture and then incubated for 1 h. After the reaction was stopped by 0.5 M $CaCl_2$ and 0.1 M trihydroxymethyl aminomethane (pH 12), the product was filtered and analyzed by an UV-VIS

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spectrophotometer (UV-1700, Shimazu) at 410 nm (Tabatabai, 1994). The measurements of NAG and PME activities were the similar to the assay of BG activity but utilized *p*-nitrophenyl-N-acetyl-β-D-glucosaminidine and *p*-nitrophenyl-phosphate as the substrates and the pH values of reaction systems were adjusted to 5.5 (Wang et al., 2015) and 6.5 (Tabatabai, 1994), respectively. The unit of BG, NAG and PME activities was express as the production of p-nitrophenol (PNP) per hour as catalyzed by 1g dry soil (mg PNP kg soil^{-1} h^{-1}).

2.4 Statistical analyses

The values of theoretical dilution were calculated based on 90%, 70%, 50%, and 30% of the measured parameters in C0 treatment (without sand addition) for C10, C30, C50, and C70 treatments, respectively. Two-way ANOVAs were conducted to determine the effects of soil coarseness and soil depth on SOC and TN contents and stocks. One-way ANOVA was executed to determine the soil coarseness effect on DOC, TDN, Olsen-P, enzyme activities, and stoichiometry of microbial biomass and enzyme activities. Multiple comparisons of Duncan's test were conducted to compare the significant difference among treatments for SOC and TN contents and stocks (run separately for each soil depth), DOC, DON, Olsen-P, enzyme activities, and stoichiometry of microbial biomass and enzyme activities. Pearson correlation analysis was executed to determine relationships between microbial biomass as well as enzyme activities and soil physicochemical properties. Multivariate linear regression analyses (stepwise removal) were used to determine parameters that made

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significant contributions to the variation of microbial biomass and enzyme activities. All statistical analyses were performed in SPSS 16.0 (SPSS, Inc., Chicago, IL, U.S.A) with $\alpha < 0.05$.

3 Results

3.1 Soil C and N pools decreased with soil coarseness and soil depth

The contents and stocks of both SOC and TN significantly decreased with soil coarseness intensities. At 0-10 cm, SOC content decreased significantly from 4.0 to 2.7 g kg soil^{-1} from no river sand addition to 70% sand addition (Fig. 1a). The TN content ranged from 0.48 to 0.22 g kg soil^{-1} and decreased significantly with soil coarseness (Fig. 1b). Both SOC and TN stocks (ranging from 6.9 to 4.4 kg m^{-3} and 0.84 to 0.38 kg m^{-3}, respectively) significantly declined with the increase of soil coarseness (Fig. 1c,d). Across all soil coarseness intensities in surface soils, soil C and N stocks decreased by as much as 31.8% and 54.0 %, respectively. At the 0-10 cm soil layer, the ratio of SOC to TN (soil C:N) varied from 8.5 to 13.1 and significantly increased with soil coarseness (Fig. S1a). For both 10-20 cm and 20-40 cm soil layers, soil coarseness significantly decreased SOC and TN contents (Fig. 1a,b). Similar trends were found for SOC and TN stocks at 10-20 cm and 20-40 cm (Fig. 1c,d). For the 10-20 cm layer, SOC and TN stocks decreased from 5.9 to 3.4 kg m^{-3} (by up to 38.2%) and 0.51 to 0.34 kg m^{-3} (by up to 25.6%) with soil coarseness (Fig. 1c). At 20-40 cm, SOC and TN stocks decreased from 4.8 to 3.3 kg m^{-3} (31.5%) and 0.52 to 0.31 kg m^{-3} (31.2%), respectively, with increasing levels of soil coarseness (Fig. 1d).

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The contents and stocks of both SOC and TN varied through soil depths (Fig. 1). Averaging all degrees of soil coarseness, SOC content, ranging from 4.0 to 1.9 mg kg soil^{-1}, was the highest at 0-10 cm and the lowest at 20-40 cm (Fig. 1a). Both TN content (Fig. 1b) and SOC stocks (Fig. 1c) followed the same trend as SOC content across the three soil depths. Surface soil (0-10 cm) retained the highest TN stocks, while no difference was detected between 10-20 cm and 20-40 cm soils (Fig. 1d).

The DOC, TDN and Olsen-P contents were analyzed in 0-10 cm soil only. The DOC content decreased significantly with increased soil coarseness from C0 (66.3 mg kg soil^{-1}) to C70 (53.9 mg kg soil^{-1}) (Fig. 2a). The TDN content was significantly lower in C50 (13.1 mg kg soil^{-1}) and C70 (12.9 mg kg soil^{-1}) as compared to C0 (14.8 mg kg soil^{-1}) (Fig. 2b). However, soil Olsen-P content was not influenced by soil coarseness (Fig. 2c).

3.2 Changes in soil microbial biomass under soil coarseness

The MBC content significantly decreased from 97.4 (in C0) to 54.5 (in C70) mg kg soil^{-1} with soil coarseness (Fig. 2d). Similarly, MBN content declined from 11.3 (in C0) to 5.4 (in C70) mg kg soil^{-1} under soil coarseness (Fig. 2e). However, MBP, ranging from 5.1 to 2.9 mg kg soil^{-1} was significantly higher in coarsened soils than that of C0 (Fig. 2f).

Soil coarseness showed no effect on the ratio of MBC to MBN (microbial C:N) (Fig. S1b). Microbial C:P significantly decreased under soil coarseness with the highest ratio of 37.8 in C0 treatment (Fig. S1c). Ranging from 4.1 (in C0) to 1.4 (in

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C50), microbial N:P ratio was also decreased significantly with soil coarseness (Fig. S1d).

3.3 Soil extracellular enzyme activities as affected by soil coarseness

The activities of BG, NAG, and PME decreased significantly with progressive soil coarseness. The BG activity decreased significantly under soil coarseness by 20.2% - 57.5% (Fig. 3a). The NAG activity varied from 6.4 mg PNP kg soil^{-1} to 13.6 mg PNP kg soil^{-1} and decreased significantly under soil coarseness by 24.5% - 53.0% (Fig. 3b). The activity of acid PME significantly decreased from 109.1 mg PNP kg soil^{-1} to 12.3 mg PNP kg soil^{-1} by 22.2% - 88.7% under soil coarseness (Fig. 3c). The BG:NAG ratio was not affected by soil coarseness (Fig. 3d). Both BG:PME and NAG:PME ratios were significantly increased in the C70 treatment (Fig. 3e,f).

3.4 Correlation between soil parameters

Soil pH significantly increased from 6.7 (C0) to 7.3 (C70) in 0-10 cm soil layer (Lü et al. 2016). The proportion of soil fine particles of < 250 μm significantly decreased from 83.1% to 39.1% with soil coarseness in 0-10 cm soil layer (Lü et al. 2016). Both MBC and MBN were significantly and positively correlated with SOC, TN, fine particles (< 250 μm), and DOC, but they were negatively correlated with soil pH (Table 1). As suggested by multiple regression models, soil fine particles accounted for 57.8% of the variation in MBC, and soil pH explained 53.3% of the variation in MBN (Table 1). A significant negative correlation was detected between

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MBP and Olsen-P, and Olsen-P explained 16% of variation in MBP (Table 1). The three enzyme activities (BG, NAG and PME) were significantly positively correlated with SOC, TN, soil fine particles, DOC, and TDN (Table 1). According to multiple regression models, TN explained 64.0% variation in BG activity, and 51.8% of variation in NAG activity (Table 1). For PME activity, 90.3% of its variation was explained by SOC, soil fine particles, and soil pH (Table 1). Soil pH was negatively correlated with SOC, TN, DOC, TDN, exchangeable Ca and Mg, and available Fe (Table 2).

3.5 The differences between theoretical dilution and the measured parameters

For SOC content, the measured values was significantly higher than the theoretical values for C30, C50, and C70 treatments in 0-10 cm soil, for C70 in 10-20 cm, and for C50 and C70 in 20-40 cm (Fig. 1a vs. Fig. 4a). As compared to theoretical dilution, significantly higher TN content values were detected in C50 and C70 treatments of 0-10 cm soil, in C30, C50, and C70 of 10-20 cm, and in C10, C30, C50, and C70 of 20-40 cm for measured parameter (Fig. 1b vs. Fig. 4b).

The DOC content was less decreased in C30, C50, and C70 treatments of field plots than that of theoretical dilution (Fig. 2a). Similarly, the TDN values of theoretical dilution group were significantly lower than that of measurement group in C10, C30, C50, and C70 treatments (Fig. 2b). The Olsen-P content significantly decreased with sand addition under theoretical dilution but not for field measurement (Fig. 2c). For microbial biomass C, N, and P, theoretical dilution showed faster decrease of these parameters (Fig. 2d,e,f). No difference was detected between

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theoretical dilution and measured parameter for both BG and NAG activities (Fig. 3a,b). However, the acid PME activity decreased faster in field plots than that of theoretical dilution in C50 and C70 treatments (Fig. 3c).

4 Discussion

4.1 The difference in values between theoretical dilution and laboratory measurements

Compared to theoretical dilution, higher measured values of SOC and TN content might be due to incorporation of external C and N sources through sand addition. Moreover, litter decomposition in field conditions might also increase SOC and TN contents as plan C input was commonly recognized as one of the main controllers of SOM content (Xiao et al., 2007). Under field condition, higher values of DOC, TDN, and Olsen-P contents could be influenced by various factors as their mobility. In this dryland ecosystem, stronger evaporation than precipitation (Nielsen and Ball, 2014) could bring these mobile C, N and P fractions from subsoil to surface soil (Luo et al., 2016) which resulted in higher measured values than theoretical dilution. Moreover, higher soil extractable C, N and P contents could be derived from plant residues in the field conditions (Halvorson et al., 2016) which was not the case for theoretical dilution. Microbial biomass and activity could be affected by plant growth (Sanaullah et al., 2011; Zhang et al., 2010) and soil physiochemical properties (Sinsabaugh et al., 2008). At presence of plant community, soil microorganisms could benefit from rhizosphere exudates or root turnover (Sanaullah et al., 2011; Wang et al., 2010), but

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might also suffer nutrient limitation from plant-microbe competitions (Dunn et al., 2006). Thus, it would be reasonable to detect higher microbial biomass C, N and P under field conditions as compared to theoretical dilution (Fig. 2d,e,f). In this study site, sand addition increased soil pH from 6.7 to 7.3 (Lü et al., 2016). Soil pH is a fundamental controller on both microbial biomass and activity (Rousk et al., 2009). Previous studies suggested that bacterial growth increased with higher soil pHs (Bååth, 1998; Rousk et al., 2009). Proliferation of soil bacteria with the increase of soil pH might be the reason of significantly higher microbial biomass C, N, and P in field condition than that of theoretical dilution (Fig. 2d,e,f). Increase of soil pH might be the reason of sharper decrease of acid PME activity in field condition as compared to theoretical dilution (Fig. 3c) with the fact that the optimal pH for acid PME activity is 6.5 (Tabatabai, 1994).

4.2 Negative effect of soil coarseness on soil C and N pools

Consistent with our hypothesis, soil C and N contents and stocks were significantly decreased by soil coarseness (Fig. 1). Soil fine particles (size of < 250 μm) are usually nutrient-rich and associated with SOM, but they are erodible during desertification (Li et al., 2004). We previously found the decline of soil fine particles during simulated soil coarseness in this field plot (Lü et al., 2016). Removal of soil fine particles by wind erosion could result in a decline of soil structure and loss of SOC and nutrients (Su et al., 2004; Lal, 2014). Our results are consistent with those of Lal (2014) and Su et al. (2004), as indicated by a significant positive correlation

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between SOC content and proportions of soil fine particles (Fig. S2). Moreover, loss of SOM could result from deterioration in soil structure (or decrease in microaggregation) and limited stabilizing effects of mineral associations after soil coarseness (Su et al., 2004). Consistent with our findings, previous studies have suggested the negative responses of soil fine particles and SOM to desertification and soil coarseness (Zhao et al., 2006; Zhou et al., 2008). Our findings of linear decrease in soil C and N under soil coarseness were in contrast to Zhou et al. (2008) who found the declines in soil C and N content was greater in light and moderate desertification stages by sampling along different natural desertification gradients. The discrepancy might be due to the differences between field manipulations and field investigations along natural gradient. Field manipulation experiments are usually precisely controlled and the results from them show clear trends, while results from investigations along natural gradient might be confounded by an array of environmental factors. The decrease of soil C and N pools from topsoils to subsoils is well-known and should be mainly due to lower C and N inputs from plant biomass and microbial residues (Wang et al., 2014).

Soil C pool is the largest terrestrial C pool and even small changes of this pool can cause significant change in atmospheric CO_2 content (Houghton et al., 1999). The reduction of soil C stocks with soil coarseness (by up to 38.2% in this study) would transfer C from soil to atmosphere which in turn would enhance the carbon-climate feedback and worsen the greenhouse effect (Duan et al., 2001; Yang et al., 2005). Our results indicated that soil N stocks of surface soil decreased faster than that of soil C

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(Fig. 1c,d). This contradicted with findings of Zhou et al. (2008) who found a greater effect of desertification on ecosystem C storage than N storage. Greater losses of ecosystem C stock relative to N resulted from decrease in soil C stock but also from decrease in grassland productivity in the study of Zhou et al. (2008). However, in this study, only soil C and N stocks were determined and thus showed greater N decrease under soil coarseness relative to C. As N constrains the productivity of most terrestrial ecosystems (Vitousek et al., 1997), soil coarseness might aggravate plant N limitation in dryland ecosystems. In this case, dryland ecosystems, which cover 41% of world land area and are prone to soil coarseness, should be better protected from further degradation.

4.3 Soil coarseness decreased soil microbial biomass and enzyme activities

Significant decrease of MBC (Fig. 2d), MBN (Fig. 2e), and extracellular enzyme activities (Fig. 3a,b,c) supported our second hypothesis, while the increase of MBP under soil coarseness (Fig. 2f) was not expected. As suggested by the correlation and regression analyses, soil physicochemical properties contribute to the changes in microbial parameters (Table 1). Given the earlier findings that enzyme activities positively correlated with soil microbial biomass, factors directly or indirectly inhibiting MBC and MBN would also suppress extracellular enzyme activities of BG, NAG and PME (Wang et al., 2014, 2015; Wolińska and Stępniewska, 2012).

Soil C is the essential element for microbial biomass production and the vital source of energetic expansion (Kemmitt et al., 2006). In this study, we observed

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positive relationships between MBC (or MBN, or enzyme activities) and SOC as well as DOC (Table 1). Based on our results, soil coarseness could possibly decrease soil microbial biomass and enzyme secretion through reduction of soil C pools (both SOC and DOC). The build-up of soil microbial biomass and secretion of enzymes (N-rich proteins) were also controlled by soil N pools (both TN and DON), especially BG and NAG activities were mostly constrained by soil TN as suggested by multiple regression models (Table 1). These findings are consistent with large-scales surveys in grassland, agricultural and forest ecosystems (Waldrop et al., 2000; Kemmitt et al., 2006; Sinsabaugh et al., 2008).

Significant correlations between soil fine particles and microbial parameters of MBC, MBN, BG, NAG and PME were found in our study (Table 1). The reduction in soil fine particles during soil coarseness might contribute to the decline in MBC and MBN. Soil coarseness, coincident with desertification and decrease of soil fine particles would provide less specific surface where microbial cells could be attached and proliferate (Van Gestel et al., 1996). At the same time, decreases in soil fine particles and smaller pore sizes expose microorganisms to predation by protozoa (Zhang et al., 2013) or to desiccation (Wang et al., 2015). With the coarseness of soil developed under desertification, fewer extracellular enzyme would be stabilized by soil minerals (Dilly and Nannipieri, 1998) resulting in decreasing of enzyme activities. Our results are in line with previous studies which showed positive relationships between microbial biomass (as well as soil enzyme activities) and the size of mineral soil particles (Kanazawa and Filip, 1986; Van Gestel et al., 1996; Wang et al., 2015).

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Soil pH is closely linked to biogeochemical processes in ecosystems and reflects the long-term plant-soil interactions and climatic variations (Kemmitt et al., 2006; Sinsabaugh et al., 2008; Rousk et al., 2009). Soil pH can strongly affect microbial growth, community composition, and activity through direct (i.e. deformation of enzyme folding and deactivation of enzyme active center) (Frankenberger and Johanson, 1982) and indirect pathways (C and nutrient availabilities and metal solubility) (Kemmitt et al., 2006). Caused by the increase of salinity from sand addition, the pH of surface soil increased nearly 0.6 units from C0 to C70 (Lü et al., 2016). As previous studies suggest that the optimal pH value for fungal growth is around pH 4.5 but above pH 7 for bacteria (Bååth, 1998; Rousk et al., 2009), the decrease of MBC as affected by soil pH might mainly result from the inhibition of fungal growth instead of bacteria (Rousk et al., 2009). Furthermore, soil pH could decrease MBC by indirectly influencing soil C and nutrient availability (Kemmitt et al., 2005, 2006), which was further indicated by the negative correlations of soil pH with SOC, TN, DOC, DON, exchangeable Ca and Mg, and available Fe contents (Table 2).

The optimal pH value for BG, NAG and PME activities are 6.0, 5.5 and 6.5, respectively (Tabatabai, 1994). Thus, the increase of soil pH from 6.7 to 7.3 with soil coarseness could reduce measured enzyme activity because the higher than optimal pH could alter functional groups of amino acids and active center of proteinic enzymes (Dick et al., 2000).

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4.4 Soil coarseness increased soil microbial C and N limitation relative to P

Soil coarseness increased the soil C:N ratio which would decrease soil nutrient (such as N and P) availability through microbial immobilization (Marschner et al., 2015). Microbial growth or activities might be constrained by C limitation as suggested by significant decrease of DOC under soil coarseness (Fig. 2a). Similarly, lower soil N availability, as partially confirmed by lower DON in this study (Fig. 2b) might result in microbial N limitation. As soil P is supplied by both biotic (SOM decomposition) and abiotic processes, unchanged Olsen-P could be balanced by the decrease of biotic release and suppression of P fixation due to decreases in clay content with increasing soil coarseness (Wang et al., 2016). This could alleviate microbial P limitation and even promote microbial P immobilization in the condition of lowering soil C:N during desertification (Marschner et al., 2015). Previous studies also suggested that soil microorganisms were capable of accumulate P in biomass even in P-depleted conditions (Chapin et al., 2002; Paul, 2014).

In this study, significant lower ratios of microbial C:P (Fig. S1c) and N:P (Fig. S1d) could possibly due to microbial accumulation of P which indicated relatively higher P availabilities relative to C and N in soils (Cleveland and Liptzin, 2007). Indeed, significant increases of BG:PME and NAG:PME ratios (Fig. 3f) suggested higher microbial C and N limitations relative to P under C70 treatment (Wang et al., 2015). In this case, plant P limitation might be enhanced due to microbial P immobilization (Xu et al., 2013). Our findings of altered microbial stoichiometry, however, suggested microorganisms did not necessarily maintain constrained element

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ratios or homeostasis like plants in response to external disturbances (Makino et al., 2003; Xu et al., 2013). These results were contrast to findings from Cleveland et al. (2007) who suggested C:N:P ratios of both soils and microorganisms were well-constrained at the global scale; while they were consistent with Sinsabaugh et al. (2008) who found ratios of microbial C-, N-, and P-acquisition enzymes were variable and depended more on environmental parameters, such as substrate availability, soil pH and the stoichiometry of microbial nutrient demand. Our work sheds light on the essential role of microbial C, N, and P ratios and enzyme ratios in understanding nutrient limitation of microbial and ecosystem processes in terrestrial ecosystem.

5 Conclusions

The significant decrease in both soil C and N pools was attributed to declines in soil fine particles during soil coarseness. For surface soils, soil TN stocks and contents decreased faster than SOC, which might increase plant N limitation in this dryland ecosystem. Soil coarseness significantly decreased soil MBC, MBN, and activities of BG, NAG and PME resulting from the decreases of soil C pools and fine particles and increases in soil pH. Enzymatic ratios, as well as microbial biomass C:N:P indicated higher microbial C and N limitation relative to P. This reflected the decrease in DOC and DON and unchanged Olsen-P content. These findings suggested microbial biomass C, N, and P and activities of C-, N-, and P-acquiring enzymes could serve as good indicators for nutrient acquisition of microorganisms and plants. Our results also imply that expansion of desertified grassland ecosystems in dry regions of the world

due to overgrazing and climate change might weaken the soil C sequestration potential and N retention capability, which in turn lead to changes in grassland productivity and biodiversity in a long run.

Author contribution

Professor X. Han designed the experiment; and L. Lü did the field work to maintain the experiment. H. Liu and X. Feng helped to do the measurement of soil parameters. R. Wang wrote the manuscript. C. Creamer helped to comment and improve the manuscript. The study was financially supported by the projects from G. Yu and Y. Jiang.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (41371251).

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Tables

Table 1 Regression statistics relating soil physicochemical properties and microbial parameters.

	SOC	TN	< 250 μm	pH	DOC	TDN	Olsen-P	Multiple
MBC	0.59	0.65	0.76**	-0.67	0.52	–	–	0.76
MBN	0.50	0.56	0.58	-0.73**	0.43	–	–	-0.73
MBP	–	–	–	–	–	–	-0.40*	-0.40
BG	0.79	0.80**	0.74	-0.73	0.73	0.54	–	0.80
NAG	0.69	0.72**	0.70	-0.68	0.60	0.45	–	0.72
PME	0.86**	0.90	0.90**	-0.88**	0.86	0.60	–	0.95

Values are R statistics for significant ($P < 0.05$) linear regressions. Multiple is R values for multiple regressions (stepwise removal) of soil physicochemical properties and microbial parameters. * and ** indicate variables that make significant contributions to the multiple linear regressions at significance level of $P < 0.05$ and 0.01, respectively.

Table 2 Relationships between soil pH and soil organic carbon (SOC), total nitrogen (TN), dissolved organic carbon (DOC), total dissolved nitrogen (TDN), exchangeable Ca (Ca^{2+}), exchangeable Mg (Mg^{2+}) and available Fe (Fe^{2+}). ** indicates significant correlation between soil parameters at $P < 0.01$.

	SOC	TN	DOC	TDN	Ca^{2+}	Mg^{2+}	Fe^{2+}
pH	-0.77**	-0.85**	-0.76**	-0.43**	-0.67**	-0.75**	-0.87**

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Figures

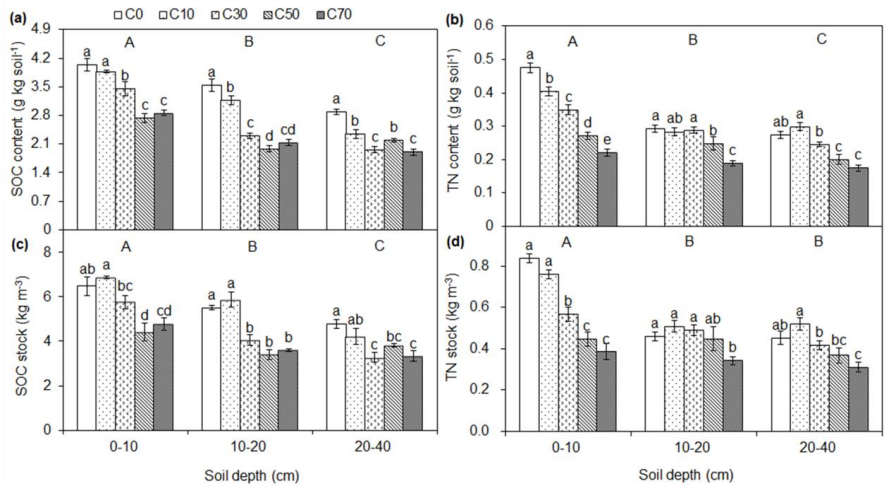

Fig. 1 Soil organic carbon (SOC) and total nitrogen (TN) contents (a and b, respectively) and stocks (c and d, respectively) as affected by different degrees of soil coarseness: 0% sand addition, (C0), 10% (C10), 30% (C30), 50% (C50), and 70% (C70) across three soil depths of 0-10 cm, 10-20 cm, and 20-40 cm. Data represent mean ± standard error (n=6). Letters indicate significant differences among treatments for each soil depth (lowercase letters) and differences among soil depths averaged across desertification degrees (capital letters).

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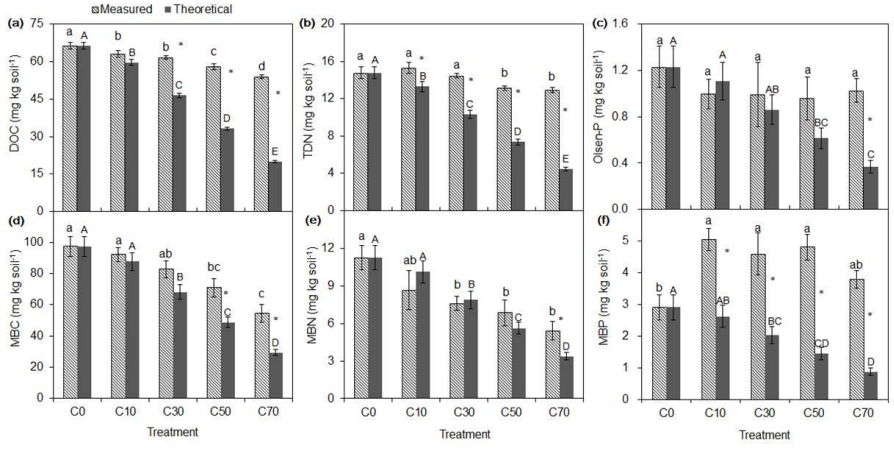

Fig. 2 Changes in soil (a) dissolved organic carbon (DOC), (b) total dissolved nitrogen (TDN), (c) Olsen phosphorus (Olsen-P), (d) microbial biomass carbon (MBC), (e) microbial biomass nitrogen (MBN), and (f) microbial biomass phosphorus (MBP) under different degrees of soil coarseness: 0% sand addition, (C0), 10% (C10), 30% (C30), 50% (C50), and 70% (C70) for 0-10 cm soil depth. Dashed bars represent values obtained from laboratory measurement, while shaded bars are values calculated from theoretical dilution. Data represent mean ± standard error (n=6). Letters indicate significant differences among treatments (lowercase letters). Asterisks indicate significance between values of laboratory measurement and theoretical dilution within one treatment.

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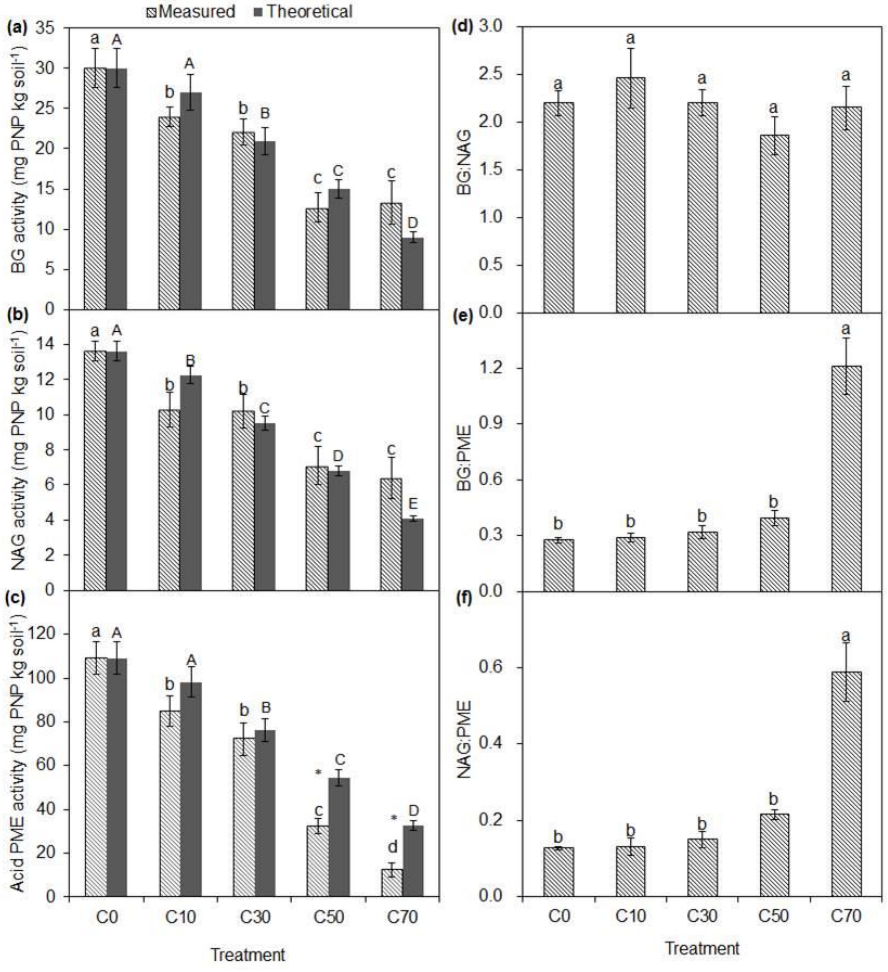

Fig. 3 Changes in (a) activities of soil β-glucosidase (BG), (b) N-acetyl-glucosaminidase (NAG), (c) acid phosphomonoesterase (PME), (d) the ratio of BG:NAG, (e) BG:PME, and (f) NAG:PME under different degrees of soil coarseness: 0% sand addition, (C0), 10% (C10), 30% (C30), 50% (C50), and 70% (C70) for 0-10 cm soil layer. Dashed bars represent values obtained from laboratory measurement, while shaded bars are values calculated from theoretical dilution. Data represent mean ± standard error (n=6). Letters indicate significant differences among treatments (lowercase letters). Asterisks indicate significance between values of laboratory measurement and theoretical dilution within one treatment.

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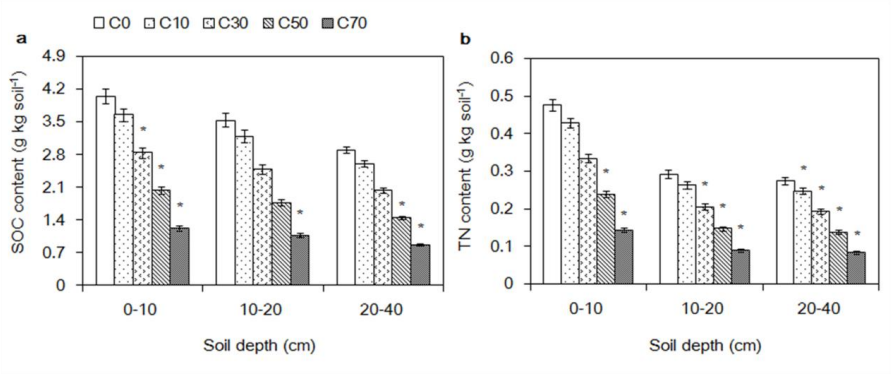

Fig. 4 Soil organic carbon (SOC) (a) and total nitrogen (TN) contents (b) as calculated from theoretical dilution for different degrees of soil coarseness: 0% sand addition, (C0), 10% (C10), 30% (C30), 50% (C50), and 70% (C70) across three soil depths of 0-10 cm, 10-20 cm, and 20-40 cm. Data represent mean ± standard error (n=6). Asterisks indicate significance between values of laboratory measurement and theoretical dilution within one treatment.