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Alteration of soil carbon and nitrogen pools and enzyme activities as affected by increased soil coarseness

Ruzhen Wang[1], Linyou Lü[1,2], Courtney A. Creamer[3], Feike A. Dijkstra[4], Heyong Liu[1], Xue Feng[1], Guoqing Yu[2], Xingguo Han[1,5], and Yong Jiang[1]

[1]State Engineering Laboratory of Soil Nutrient Management, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China
[2]Institute of Sandy Land Improvement and Utilization, Liaoning Academy of Agricultural Sciences, Fuxin 123000, China
[3]US Geological Survey, Menlo Park, CA 94025-3561, USA
[4]Centre for Carbon, Water and Food, School of Life and Environmental Sciences, The University of Sydney, Sydney, NSW 2006, Australia
[5]State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

Correspondence to: Yong Jiang (jiangyong@iae.ac.cn)

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Abstract. Soil coarseness decreases ecosystem productivity, ecosystem carbon (C) and nitrogen (N) stocks, and soil nutrient contents in sandy grasslands subjected to desertification. To gain insight into changes in soil C and N pools, microbial biomass, and enzyme activities in response to soil coarseness, a field experiment was conducted by mixing native soil with river sand in different mass proportions: 0, 10, 30, 50, and 70 % sand addition. Four years after establishing plots and 2 years after transplanting, soil organic C and total N concentrations decreased with increased soil coarseness down to 32.2 and 53.7 % of concentrations in control plots, respectively. Soil microbial biomass C (MBC) and N (MBN) declined with soil coarseness down to 44.1 and 51.9 %, respectively, while microbial biomass phosphorus (MBP) increased by as much as 73.9 %. Soil coarseness significantly decreased the enzyme activities of β-glucosidase, N-acetyl-glucosaminidase, and acid phosphomonoesterase by 20.2–57.5 %, 24.5–53.0 %, and 22.2–88.7 %, used for C, N and P cycling, respectively. However, observed values of soil organic C, dissolved organic C, total dissolved N, available P, MBC, MBN, and MBP were often significantly higher than would be predicted from dilution effects caused by the sand addition. Soil coarseness enhanced microbial C and N limitation relative to P, as indicated by the ratios of β-glucosidase and N-acetyl-glucosaminidase to acid phosphomonoesterase (and MBC : MBP and MBN : MBP ratios). Enhanced microbial recycling of P might alleviate plant P limitation in nutrient-poor grassland ecosystems that are affected by soil coarseness. Soil coarseness is a critical parameter affecting soil C and N storage and increases in soil coarseness can enhance microbial C and N limitation relative to P, potentially posing a threat to plant productivity in sandy grasslands suffering from desertification.

1 Introduction

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) constraining terrestrial net primary productivity (NPP) and ecosystem health (Lal, 2014; Lü et al., 2016). Currently, more than 30 % of world total dryland area and 0.85 billion people are directly influenced by desertification and soil coarseness (Zhou et al., 2008; Chang et al., 2015). It has become increasingly clear that desertification and soil coarseness cause reductions in NPP (Peters et al., 2012), soil organic carbon (SOC) storage (Zhou et al., 2008), and nutrient retention (Delgado-Baquerizo et al., 2013). These effects of desertification and soil coarseness pose threats to world food security (Zhao et al., 2006), enhance the carbon–climate feedback (Lal, 2014), and 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 C generally comprises 1–4 % of soil organic C, but it substantially contributes to stable soil C formation and 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 (Richardson and Simpson, 2011). Soil nutrient supply is predominately controlled by microbial decomposition of soil organic matter (SOM) (although P can also be supplied through 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, microbial mineralization of SOM is often constrained by C and nutrient availabilities (Cleveland et al., 2002), as well as by enzymatic stoichiometry and kinetics (Sinsabaugh et al., 2008, 2014; Wang et al., 2015). For instance, microbial P limitation decreased SOM decomposition in tropical soils (Cleveland et al., 2002). In both tropical and temperate soils, lower ratios of soil β-glucosidase (BG) to acid phosphatase (PME) and N-acetyl-glucosaminidase (NAG) to PME were observed, illustrating greater microbial P demand relative to C and N, respectively (Waring et al., 2014; Wang et al., 2015). Although a large number of studies have investigated desertification and soil coarseness effects on plant productivity (Zhao et al., 2006), soil C and N dynamics (Zhou et al., 2008), and soil nutrient availability to plants (Zhao et al., 2006; Li et al., 2013), studies on microbial biomass C, N and P, soil enzyme activities, and microbial nutrient limitations are still rare. Soil coarseness effects on 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 components of the Inner Mongolian grassland system belonging to the Eurasian steppe. The southeastern edge of the Horqin Sandy Grassland used to be a productive steppe grassland until the 1950s, when overgrazing and over-cultivation occurred to support the 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 low plant cover and high annual wind speed (varying from 3.4 to 4.1 $\mathrm{m\,s^{-1}}$) with frequent occurrence of gales (wind speed > 20 $\mathrm{m\,s^{-1}}$) (Lü et al., 2016). Natural gradients and long-term monitoring studies have been used to examine desertification (Zhao et al., 2006; Zhou et al., 2008), but they do not control for climatic parameters, such as temperature, precipitation, and solar radiance. Therefore, controlled field experiments are necessary (i.e., treatments with similar initial soil type and climatic factors) to better understand mechanisms of desertification caused by soil coarseness. In our previous work we showed that soil pH, fraction of soil fine particles (< 250 µm), soil exchangeable Ca and Mg, and soil available Fe were significantly lower with increased soil coarseness (Lü et al., 2016). In this study, we hypothesized that (1) soil coarseness would decrease both soil C and N concentrations 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; and (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 the town of Zhanggutai (42°43′ N, 122°22′ E; elevation 226.5 m a.s.l.) at the southeast of the Horqin Sandy Grassland of northern China. The mean annual temperature is 6.3 °C and the mean annual precipitation is 450 mm. The soil at this site is sandy with a bulk density of 1.66 $\mathrm{g\,cm^{-3}}$ and containing 4.04 $\mathrm{g\,kg^{-1}}$ SOC and 0.48 $\mathrm{g\,kg^{-1}}$ total N (TN). The soil is an Aeolic Eutric Arenosol in the FAO classification (IUSS Working Group WRB, 2014).

In May 2011, treatments were established in 4 m × 4 m plots arranged in a complete randomized design with five treatments and six replicates (Fig. 1a). Original plants were removed before preparing the soil. To simulate different degrees of soil coarseness, soils from three soil depths (0–20, 20–40, and 40–60 cm) were dug out and evenly mixed with 2 mm sieved river sand in different mass proportions and then refilled back. By keeping the total mass constant to the control soil, we mixed different mass proportions of 0 (C0), 10 (C10), 30 (C30), 50 (C50), and 70 % (C70) sand with soil. Therefore, we had in total five sand addition treatments. The river sand contained $1.29 \pm 0.04\ \mathrm{g\,kg^{-1}}$ C and $0.15 \pm 0.03\ \mathrm{g\,kg^{-1}}$ N with a pH of 7.5 ± 0.2. In August 2012, soils at 0–5 cm depth were taken out from all plots and sterilized at 105 °C for 3 h to deactivate the seeds and prevent plant growth (Fig. 1b). The 0–5 cm soils were filled back and all plots were equilibrated for 1 year. Control plots without sand addition (C0) were subjected to the same method of sterilization. We did not include a control treatment with original soil and without sterilization, which would have allowed us to assess the effect of alteration in soil structure and physicochemical parameters caused by the sterilization method on biogeochemical processes. In July 2013, native plant species were transplanted from a local grassland in abundances sim-

Figure 1. Overview of field experimentation with photos taken at various stages of the experiment.

ilar to the native community composition (Fig. 1c). Before transplanting, roots of the plants were gently washed to remove native soil. Through soil sterilization and transplanting, we established plots that started with the same plant community similar to the nearby native grassland. Since 2014, a permanent area of 1 m × 1 m was set up within each plot to investigate plant community composition annually (in August) (Fig. 1d). Precipitation data from 2014 to 2016 were collected from the weather station located near the field site.

2.2 Soil sampling and chemical analysis

In October 2015, soil samples at 0–10 cm depth were taken by compositing three randomly placed soil cores within each plot (Fig. 1e). Fresh samples were passed through a 2 mm sieve, sealed in plastic bags, and stored at 4 °C until further processing.

The concentrations of SOC and TN were determined in 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 were calculated by multiplying SOC or TN concentrations by soil bulk density. The soil dissolved organic C (DOC) and total dissolved N (TDN) were extracted from 15 g fresh soils with 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 concentrations of DOC and TDN in filtrate were determined by a TOC analyzer (Multi N/C 3100, Analytik Jena, Germany).

Soil pH was measured in a 1 : 2.5 (w/v) soil–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. The fraction of soil fine particles $< 250\,\mu m$ was calculated as the sum of fine sand, silt, and clay fraction. Soil exchangeable Ca and Mg were extracted with a 1 M CH_3COONH_4 solution (Ochoa-Hueso et al., 2014). Available Fe was extracted with diethylenetriaminepentaacetic acid (DTPA) (Lindsay and Norvell 1978). Soil exchangeable Ca and Mg and available Fe were analyzed with an atomic absorption spectrometer (AAS, Shimadzu, Japan). Soil water-holding capacity was determined on air-dried soils according to Wang et al. (2017).

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). 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 was mixed with 60 mL 0.5 M $NaHCO_3$ and shaken at 150 rpm for 1 h. After filtration, the extractable P concentration in the filtrate was determined with the molybdenum blue colorimetric method (Murphy and Riley, 1962). The measured P concentration in unfumigated soil samples is referred to as Olsen-P (Wang et al., 2016). To correct for incomplete extraction, we used efficiency factors of 0.45, 0.54, and 0.40 to calculate the actual concentrations 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 field moist soil samples. All soil samples were frozen in the same way to minimize potential freezing effects on enzyme activities (Razavi et al., 2016). For BG activity, p-nitrophenyl-β-D-glucopyranoside (0.05 M) was added as the indicator substrate and the product from the enzyme assay was analyzed with a UV–visible spectrophotometer (UV-1700, Shimazu) at 410 nm (Tabatabai, 1994). The measurements of NAG and PME activities were similar to the assay of BG activity but with p-nitrophenyl-N-acetyl-β-D-glucosaminidine and p-nitrophenyl-phosphate as the substrates, respectively. The pH values of the reaction systems for NAG and PME were adjusted to 5.5 (Wang et al., 2015) and 6.5 (Tabatabai, 1994), respectively. The activities of BG, NAG, and PME were expressed as production rates of p-nitrophenol (PNP, in mg PNP kg soil^{-1} h^{-1}).

2.4 Statistical analyses

We calculated the theoretical dilution in SOC and TN caused by the addition of river sand. Because the river sand contained small amounts of total C and N, the values of theoretical dilution were calculated based on mass proportions of sand and soil by considering the concentrations of SOC and TN in both sand and the C0 treatment (without sand addition) for the C10, C30, C50, and C70 treatments, respectively. For other soil parameters, values of theoretical dilution were calculated as 90, 70, 50, and 30 % of the measured parameters in the C0 treatment (without sand addition) for the C10, C30, C50, and C70 treatments, respectively. One-way ANOVA was conducted to determine the effects of soil coarseness on SOC and TN concentrations and stocks, concentrations of DOC, TDN and Olsen-P, enzyme activities, and stoichiometry of microbial biomass and enzyme activities. Multiple comparisons of Duncan's test were conducted to compare the significant differences among treatments for SOC and TN concentrations and stocks, DOC, TDN, Olsen-P, enzyme activities, and stoichiometry of microbial biomass and enzyme activities. Pearson correlation analysis was performed 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 significant contributions to the variation of microbial biomass and enzyme activities. All statistical analyses were performed in SPSS 16.0 (SPSS, Inc., Chicago, IL, USA) with $\alpha < 0.05$.

3 Results

3.1 Soil moisture, water-holding capacity, and soil C and N pools with soil coarseness

The annual precipitation was 383.8, 419.5, and 615.9 mm in 2014, 2015, and 2016, respectively (Fig. 2a). Precipitation in 2014 and 2015 was below and in 2016 above the mean annual precipitation from long-term records (450 mm). Soil moisture in samples taken in October 2015 decreased with increasing soil coarseness from 10.6 to 6.8 % ($P < 0.01$, Fig. 2b). Soil coarseness also decreased soil water-holding capacity in the C50 and C70 treatments compared to the C0 treatment ($P < 0.01$, Fig. 2c).

The concentrations and stocks of both SOC and TN decreased with increasing soil coarseness. The SOC concentration decreased from 4.0 to 2.7 g kg soil^{-1} from in the C0 to the C70 treatment ($P < 0.01$, Fig. 3a). The TN content ranged from 0.48 to 0.22 g kg soil^{-1} and also decreased with increased soil coarseness ($P < 0.01$, Fig. 3b). Both SOC and TN stocks declined with increased soil coarseness ($P < 0.01$, Fig. 3c, d). Across all soil coarseness levels, soil C and N stocks decreased by as much as 31.8 and 54.0 %, respectively. The ratio of SOC to TN (soil C : N) increased with increased soil coarseness ($P < 0.01$, Fig. S1a). The DOC concentration decreased with increased soil coarseness ($P < 0.01$, Fig. 4a). The TDN concentration was lower in the C50 and C70 treatment compared to the C0 treatment ($P = 0.002$, Fig. 4b). However, soil Olsen-P content was not influenced by soil coarseness ($P = 0.84$, Fig. 4c).

3.2 Changes in soil microbial biomass under soil coarseness

The MBC decreased from 97.4 (in C0) to 54.5 (in C70) mg kg soil^{-1} with increased soil coarseness ($P < 0.01$, Fig. 4d). Similarly, MBN concentration declined from 11.3 (in C0) to 5.4 (in C70) mg kg soil^{-1} with increased soil coarseness ($P = 0.007$, Fig. 4e). However, MBP, ranging from 5.1 to 2.9 mg kg soil^{-1}, was higher in all treatments with sand addition than in the C0 treatment ($P = 0.012$, Fig. 4f).

Soil coarseness showed no effect on the ratio of MBC to MBN (microbial C : N) ($P = 0.64$, Fig. S1b). Microbial C : P decreased with increased soil coarseness with the highest ratio of 37.8 in the C0 treatment ($P = 0.003$, Fig. S1c). The microbial N : P ratio also decreased with increased soil coarseness, ranging between 4.1 (in C0) and 1.4 (in C50) ($P < 0.01$, Fig. S1d).

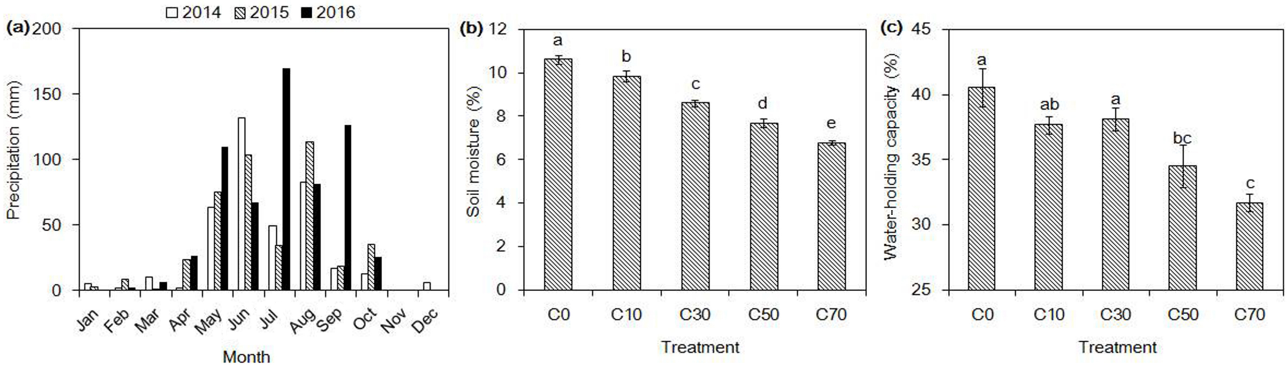

Figure 2. Monthly precipitation from 2014 to 2016 **(a)** and response of moisture **(b)** and water-holding capacity **(c)** in soil samples taken in October 2015 to different degrees of soil coarseness: 0 % sand addition (C0), 10 (C10), 30 (C30), 50 (C50), and 70 % (C70). Data represent mean ± standard error ($n = 6$). Letters indicate significant differences among treatments.

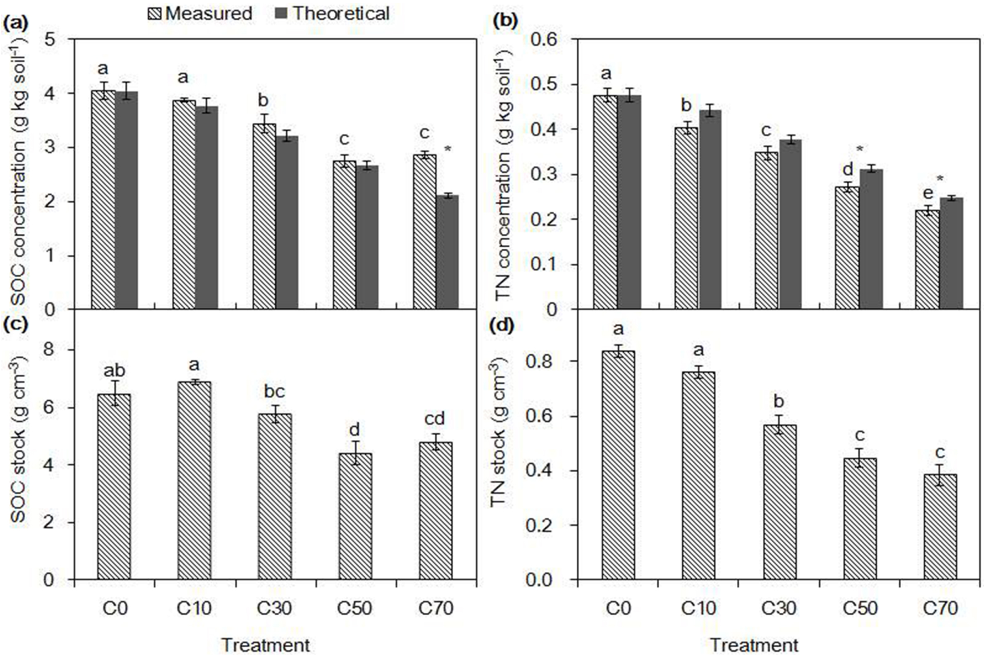

Figure 3. Soil organic carbon (SOC) and total nitrogen (TN) concentrations (**a** and **b**, respectively) and stocks (**c** and **d**, respectively) as affected by different degrees of soil coarseness: 0 % sand addition (C0), 10 (C10), 30 (C30), 50 (C50), and 70 % (C70). Dashed bars represent values obtained from laboratory measurements, while black bars are values calculated from theoretical dilution. Data represent mean ± standard error ($n = 6$). Letters indicate significant differences among treatments. Asterisks indicate significance between values from laboratory measurements and theoretical values accounting for dilution within one treatment.

3.3 Soil extracellular enzyme activities as affected by soil coarseness

The activities of BG, NAG, and PME decreased significantly with increased soil coarseness. The BG activity decreased with soil coarseness by 20.2 to 57.5 % (Fig. 5a). The NAG activity varied from 6.4 to 13.6 mg PNP kg soil^{-1} and decreased with soil coarseness by 24.5 to 53.0 % (Fig. 5b). The activity of acid PME decreased from 109.1 to 12.3 mg PNP kg soil^{-1}, or by 22.2 to 88.7 % with increased soil coarseness (Fig. 5c). The BG : NAG ratio was not affected by soil coarseness ($P = 0.41$, Fig. 5d). Both BG : PME and NAG : PME ratios were highest in the C70 treatment (both $P < 0.01$, Fig. 5e, f).

3.4 Correlation between soil parameters

Soil pH significantly increased from 6.7 (C0) to 7.3 (C70) in the 0–10 cm soil layer (Lü et al., 2016). The fraction of fine particles < 250 µm significantly decreased from 83.1 to

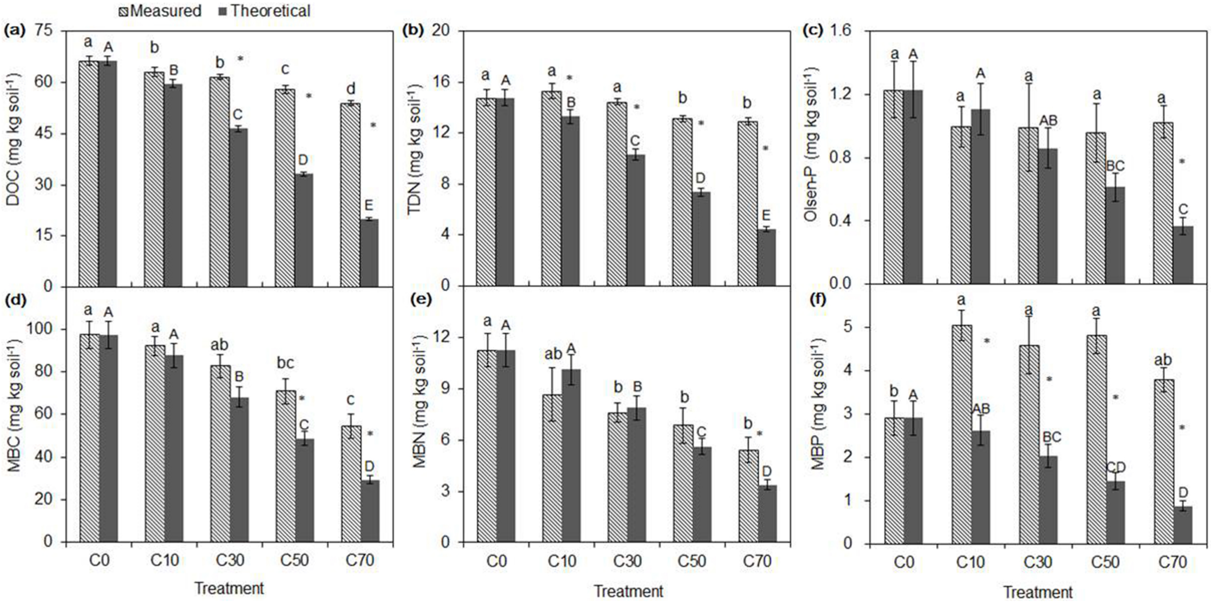

Figure 4. Changes in soil **(a)** dissolved organic carbon (DOC), **(b)** total dissolved nitrogen (TDN), **(c)** Olsen phosphorus (Olsen-P), **(d)** microbial biomass carbon (MBC), **(e)** microbial biomass nitrogen (MBN), and **(f)** microbial biomass phosphorus (MBP) as affected by different degrees of soil coarseness: 0 % sand addition (C0), 10 (C10), 30 (C30), 50 (C50), and 70 % (C70). Dashed bars represent values obtained from laboratory measurements, while black bars are values calculated from theoretical dilution. Data represent mean ± standard error ($n = 6$). Letters indicate significant differences among treatments. Asterisks indicate significance between values from laboratory measurements and theoretical values accounting for dilution within one treatment.

39.1 % with soil coarseness in the 0–10 cm soil layer (Lü et al., 2016). Both MBC and MBN were significantly and positively correlated with SOC, TN, fine particles ($< 250\,\mu$m), 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 MBP and Olsen-P, and Olsen-P explained 16 % of the variation in MBP (Table 1). The three enzyme activities (BG, NAG, and PME) were positively correlated with SOC, TN, soil fine particles, DOC, and TDN (Table 1). According to multiple regression models, TN explained 64.0 % of the variation in BG activity, and 51.8 % of the 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 measured parameters

We calculated what the theoretical concentrations in soil parameters were only accounting for the dilution effect of adding river sand. Because the river sand contained low concentrations of total C and N ($1.29 \pm 0.04\,\mathrm{g\,kg^{-1}}$ C and $0.15 \pm 0.03\,\mathrm{g\,kg^{-1}}$ N), total C and N contents in the river sand were considered when calculating the theoretical concentrations of SOC and TN.

The measured SOC concentration in the C70 treatment was significantly higher than the theoretical concentration ($P < 0.01$, Fig. 3a). However, measured TN concentrations were lower than theoretical concentrations in the C50 ($P = 0.01$) and C70 ($P = 0.03$) treatments (Fig. 3b).

The DOC concentrations in the C30, C50, and C70 treatments decreased less than expected accounting for theoretical dilution (Fig. 4a). Similarly, the theoretical TDN concentrations in the C10, C30, C50, and C70 treatments were lower than measured ($P < 0.05$, Fig. 4b). The theoretical Olsen-P concentrations decreased with increased soil coarseness ($P < 0.01$), but not the measured concentrations ($P = 0.84$) (Fig. 4c). For microbial biomass C, N, and P, measured values decreased less with increased soil coarseness than expected when accounting for theoretical dilution (Fig. 4d, e, f). No difference was detected between theoretical and measured activities for both BG and NAG (Fig. 5a, b). However, the acid PME activity decreased more strongly than the theoretical activity in the C50 and C70 treatments (Fig. 5c).

4 Discussion

In this study, we added sand at different levels to plots to mimic desertification effects on soil biogeochemical processes in a semiarid grassland in northern China. Because

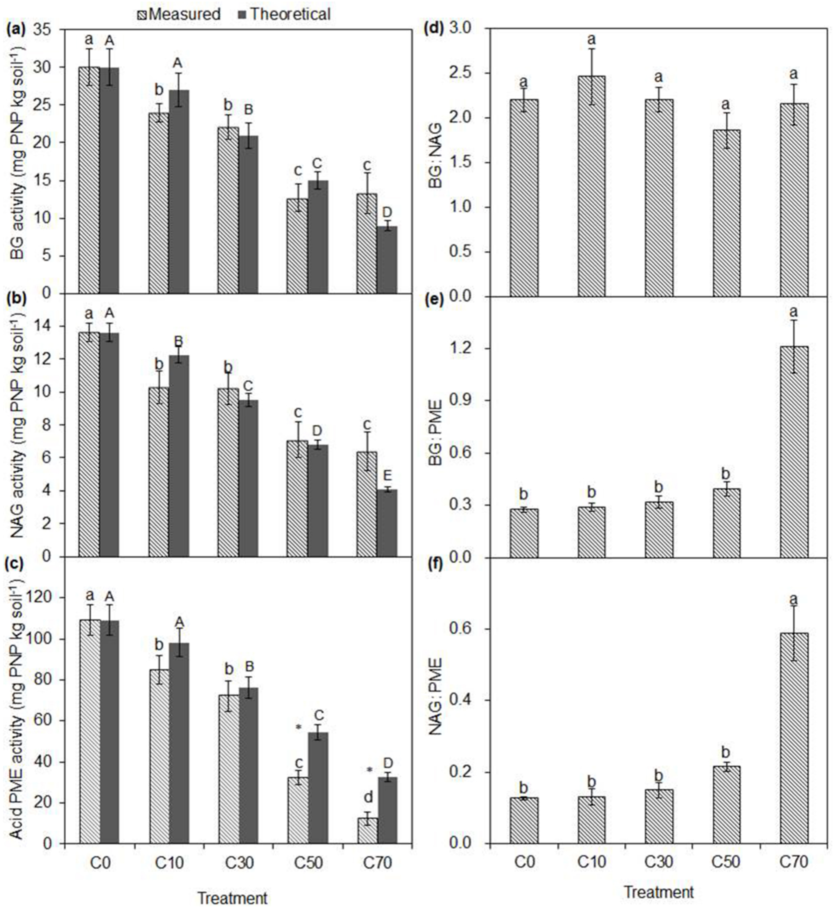

Figure 5. Changes in **(a)** activities of soil β-glucosidase (BG), **(b)** N-acetyl-glucosaminidase (NAG), **(c)** acid phosphomonoesterase (PME), **(d)** the ratio of BG : NAG, **(e)** BG : PME, and **(f)** NAG : PME as affected by different degrees of soil coarseness: 0 % sand addition (C0), 10 (C10), 30 (C30), 50 (C50), and 70 % (C70). Dashed bars represent values obtained from laboratory measurements, while black bars are values calculated from theoretical dilution. Data represent mean ± standard error ($n = 6$). Letters indicate significant differences among treatments. Asterisks indicate significance between values from laboratory measurements and theoretical values accounting for dilution within one treatment.

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

When significant ($P < 0.05$), R values of linear and multiple regressions are shown. For multiple regressions (Multiple), significant contributions of soil physicochemical properties and microbial parameters are indicated with * $P < 0.05$ and ** $P < 0.01$, after stepwise removal of non-significant parameters.

sand addition also resulted in dilution of the soil parameters we measured, we compared observed soil parameters with theoretical values we would expect if sand addition only caused a dilution effect. Therefore, differences between measured and theoretical soil parameters are caused by effects of soil coarseness other than dilution effects.

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+}).

	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*

* Indicates significant correlation between soil parameters at $P < 0.01$.

4.1 The difference between measured and theoretical soil parameters accounting for dilution effects

Compared to theoretical values accounting for dilution effects, we observed higher measured values of SOC in the C70 treatment. This could be due to increased plant C input through litter decomposition, which is commonly recognized as one of the main controllers of SOM concentration (Xiao et al., 2007). Plant uptake and N leaching processes might have contributed to the lower soil TN (measured) in the C50 and C70 treatments compared to theoretical values accounting for dilution effects (Fig. 3b).

Under field conditions, higher values of DOC, TDN, and Olsen-P compared with theoretical accounting for dilution could be influenced by various factors. In this dryland ecosystem, greater evaporation than precipitation (Nielsen and Ball, 2014) could bring these mobile C, N and P fractions from the subsoil to the surface soil (Luo et al., 2016), which could have been stimulated with soil coarseness. Moreover, higher soil extractable C, N, and P concentrations could be derived from plant residues in the field (Halvorson et al., 2016), which was not accounted for by the theoretical dilution. Microbial biomass and activity could be affected by plant growth (Sanaullah et al., 2011; Zhang et al., 2010) and soil physicochemical properties (Sinsabaugh et al., 2008). With plants present, soil microorganisms could benefit from rhizosphere exudates or root turnover (Sanaullah et al., 2011; Wang et al., 2010) but might also suffer from nutrient limitation caused by plant–microbe competition (Dunn et al., 2006). Although plant-derived deposits may have decreased with increased coarseness, this decrease may have been less than the dilution factor caused by sand addition, so that observed microbial parameters were larger than theoretically predicted (Fig. 4d).

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). The increase in soil pH might be the reason for a sharper decrease in acid PME activity with increased coarseness compared to the theoretical activity accounting for dilution (Fig. 5c), given that the optimal pH for acid PME activity is around 6.5 (Tabatabai, 1994). Absorption of acid PME by clay particles could also inhibit its activity (Dilly and Nannipieri, 1998), but this would not explain the greater decrease in measured acid PME activity with increased coarseness compared to the theoretical activity accounting for dilution.

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

Consistent with our hypothesis, soil C and N concentrations and stocks significantly decreased with increased soil coarseness (Fig. 3). Soil fine particles ($< 250\,\mu m$) are usually nutrient-rich and associated with SOM, but they are erodible during desertification (Li et al., 2004). We previously found that the decrease in soil fine particles was mainly a result of sand dilution in this field experiment (Lü et al., 2016). Removal of soil fine particles by wind erosion could result in a deterioration 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 between SOC concentration and the fraction of soil fine particles (Fig. S2). Moreover, loss of SOC could result from limited stabilizing effects of mineral associations with increased soil coarseness (Su et al., 2004). The disturbance associated with the sand addition may further have resulted in some aggregate-protected or occluded SOC becoming more available to microbial degradation. Our findings of a linear decrease in soil C and N with increased soil coarseness are in contrast to Zhou et al. (2008), who found that declines in soil C and N concentrations were greater at light and moderate desertification stages as compared to later stages along different natural desertification gradients. The discrepancy might be due to the differences between field manipulations and field investigations along a natural gradient. Because our manipulative field experiment was conducted under somewhat artificial conditions (e.g., soil disturbance and sterilization), we caution with relating our results to natural pristine ecosystems. However, one of the advantages of our manipulative experiment is that it was done under controlled conditions, allowing for a better understanding of the mechanisms of alterations in soil C and N pools and enzyme activities caused by soil coarseness.

The soil C pool is the largest terrestrial C pool and even small changes in this pool can cause significant changes in the atmospheric CO_2 concentration (Houghton et al., 1999). Our results indicated that N stocks in the surface soil decreased more than that of soil C (54.0 % vs. 31.8 %, Fig. 2c, d). This is in contradiction to the findings by Zhou et al. (2008), who found a greater effect of desertification on ecosystem C storage than N storage. Greater losses of the ecosystem C stock relative to N resulted from a decrease in the soil C stock, but also from a decrease in grassland productivity in the study of Zhou et al. (2008). However, in the current study, only soil C and N stocks were determined showing a larger N decrease with increased soil coarseness relative to C. As N constrains the productivity of most terrestrial ecosystems (Vitousek et al., 1997), soil coarseness would 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 decreases in MBC (Fig. 4d), MBN (Fig. 4e), and extracellular enzyme activities (Fig. 5a, b, c) supported our second hypothesis, while the increase in MBP under soil coarseness (discussed below, Sect. 4.4) (Fig. 4f) was not expected. As suggested by the correlation and regression analyses, soil physicochemical properties contributed 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 decreasing MBC and MBN would also suppress the synthesis of extracellular enzymes of BG, NAG, and PME (Wang et al., 2014, 2015; Wolińska and Stępniewska, 2012).

Soil C is essential for microorganisms and a vital source of growth (Kemmitt et al., 2006). In this study, we observed 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 TDN); in particular, BG and NAG activities were mostly constrained by soil TN as suggested by multiple regression models (Table 1). These findings are consistent with large-scale surveys in grassland, agricultural, and forest ecosystems (Waldrop et al., 2000; Kemmitt et al., 2006; Sinsabaugh et al., 2008).

Significant correlations between the fraction of soil fine particles and microbial parameters of MBC, MBN, BG, NAG, and PME were found in our study (Table 1). The reduction in the fraction of soil fine particles with increased soil coarseness might have contributed to the decline in MBC and MBN. Soil coarseness, associated with desertification and a decrease in the fraction of soil fine particles, would provide less specific surface area to which microbial cells could attach and proliferate (Van Gestel et al., 1996). At the same time, decreases in the fraction of soil fine particles and smaller pore sizes expose microorganisms to predation by protozoa (Zhang et al., 2013) or to desiccation (Alster et al., 2013). Although it was not extremely dry during the year when soil samples were taken (2015) (Fig. 2a), a significant decrease in soil moisture (Fig. 2b) and water-holding capacity (Fig. 2c) indicated that soil microorganisms were exposed to drier conditions with increased soil coarseness. Drier conditions would result in lower microbial activity and secretion of extracellular enzymes (Wang et al., 2014). With increased soil coarseness due to desertification, fewer extracellular enzyme could be stabilized by soil minerals (Dilly and Nannipieri, 1998), resulting in decreasing 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).

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 the enzyme active center) (Frankenberger and Johanson, 1982) and indirect pathways (affecting C and nutrient availabilities and metal solubility) (Kemmitt et al., 2006). Because the added sand was more alkaline than the soil, the pH of the surface soil increased nearly 0.6 units from the C0 to the C70 treatment (Lü et al., 2016). Soil pH could decrease MBC indirectly by influencing soil C and nutrient availability (Kemmitt et al., 2005, 2006), which was also indicated by the negative correlations of soil pH with SOC, TN, DOC, TDN, exchangeable Ca and Mg, and available Fe (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 in soil pH from 6.7 to 7.3 with soil coarseness may have reduced the enzyme activities affecting the functional groups of amino acids and active center of enzymes (Dick et al., 2000).

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

Soil coarseness increased the soil C : N ratio, which may result in decreased soil nutrient (such as N and P) availability through microbial immobilization (Marschner et al., 2015). Microbial growth or activities could be constrained by C limitation as suggested by the significant decrease in DOC with increased soil coarseness (Fig. 4a). Similarly, lower soil N availability, as partially confirmed by lower TDN in this study (Fig. 4b), might result in microbial N limitation. In contrast, Olsen-P was not affected by soil coarseness. Possibly, a decrease in SOM decomposition (and P mineralization) may have been counterbalanced by a net increase in abiotic supply of P due to suppression of P fixation associated with a lower clay content with increasing soil coarseness (Wang et al., 2016). This could alleviate microbial P limitation and even promote microbial P immobilization with lower soil C : N caused by desertification (Marschner et al., 2015). Previous studies have also suggested that soil microorganisms were capable of accumulating P in biomass even under Pdepleted conditions (Chapin et al., 2002; Paul, 2014). Thus, the third hypothesis was supported by our data.

In this study, significantly lower ratios of microbial C : P (Fig. S1c) and N : P with increased soil coarseness (Fig. S1d), possibly due to microbial accumulation of P, suggest higher microbial P availability relative to C and N in soils (Cleveland and Liptzin, 2007). Indeed, significant increases in the BG : PME and NAG : PME ratios (Fig. 4f) suggest higher microbial C and N limitations relative to P in the C70 treatment (Wang et al., 2015). In this case, plant P limitation might be alleviated due to microbial P immobilization (Xu et al., 2013), because microbial biomass turnover and P re-mobilization from MBP would increase P availability to plants in the medium and long term. Our findings of altered microbial stoichiometry, however, suggest that microorganisms did not necessarily maintain fixed elemental ratios (or maintain stoichiometric homeostasis) like plants in response to external disturbances (Makino et al., 2003; Xu et al., 2013). These results were in contrast to findings from Cleveland et al. (2007), who suggested that C : N : P ratios of both soils and microorganisms were well constrained at the global scale. However, our results were consistent with Sinsabaugh et al. (2008), who found that 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.

Overall, by mixing soil with sand in different mass proportions to simulate various levels of soil coarseness as affected by desertification, the results imply that desertification aggravates water limitation to plants and soil microorganisms as indicated by decreased soil moisture and water-holding capacity in this semi-arid grassland. Soil moisture has proven to be the key parameter influencing soil nutrient mobilization and microbial biomass and activity in this water-limited ecosystem. Also, desertification would decrease soil C and N stocks as well as soil C, N, and P cycling rates as suggested by lower extracellular enzyme activities. 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 ecosystems subjected to desertification.

5 Conclusions

The significant decrease in both soil C and N pools was attributed to declines in the fraction of soil fine particles with increased soil coarseness. Soil TN stocks and concentrations decreased more 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 in soil moisture, C pools and fraction of 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 was also reflected in the decreased DOC and TDN and unchanged Olsen-P concentration with increased soil coarseness. These findings suggest that 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 would decrease the soil C and N stocks, which in turn lead to changes in grassland productivity and biodiversity in the long run.

Data availability. Data sets for this paper can be obtained via personal communication.

The Supplement related to this article is available online at doi:10.5194/bg-14-2155-2017-supplement.

Author contributions. Xingguo Han designed the experiment, and L. Lü did the field work to maintain the experiment. Heyong Liu and Xue Feng helped with the measurements of soil analyses. R. Wang wrote the manuscript. Courtney A. Creamer and Feike A. Dijkstra helped to improve the manuscript. The study was financially supported by the projects from Guoqing Yu and Yong Jiang.

Competing interests. The authors declare that they have no conflict of interest.

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