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

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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 two 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.

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

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

5   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,

10   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

15   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

20   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 m s^{-1}) with frequent occurrence of gales (wind speed > 20 m

5   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

10 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

15 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.

20 **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 the Horqin Sandy Grassland of northern

China. The mean annual temperature is 6.3 ℃ and the mean annual precipitation is

450 mm. The soil at this site is sandy with a bulk density of 1.66 g cm^{-3} and

containing 4.04 g kg^{-1} SOC and 0.48 g kg^{-1} total N (TN). The soil is an Aeolic Eutric

5   Arenosol in the FAO classification (IUSS Working Group WRB, 2014).

In May 2011, treatments were established in 4 m $\times$ 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 cm, 20-40 cm, and 40-60 cm) were dug

10   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$0.04 g kg^{-1} C and 0.15$\pm$0.03 g kg^{-1} N with a pH of 7.5$\pm$0.2.

15   In August 2012, soils at 0-5 cm depth were taken out from all plots and sterilized at

105 ℃ for 3h 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

20   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

similar 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 ℃ 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 with 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, 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. The

fraction of soil fine particles < 250 μm was calculated as the sum of fine sand, silt and

5   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, Shimazu, Japan). Soil water holding capacity was determined on

10   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

15   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

20   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

5 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 an UV-VIS 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 now we used

10 *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

20 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

5   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 executed to determine relationships between

10   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, U.S.A) 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 mm, 419.5 mm and 615.9 mm in 2014, 2015

and 2016, respectively (Fig. 2a). Precipitation in 2014 and 2015 were below and in

20   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% down 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 content 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) to 1.4 (in

C50) ($P < 0.01$, Fig. S1d).

5   *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

10   from 109.1 mg 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).

15   *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 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

20   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 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 ± 0.04 g kg^{-1} C and 0.15 ± 0.03 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

5   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

10   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 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

15   and theoretical soil parameters are caused by effects of soil coarseness other than

dilution effects.

4.1 The difference between measured and theoretical soil parameters accounting for

dilution effects

       Compared to theoretical values accounting for dilution effects, we observed

20   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 content (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 contents 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 physiochemical 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 of 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 μm) are usually nutrient-rich and associated with SOM, but they are erodible

10   during desertification (Li et al., 2004). We previously found that the decrease of 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

15   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

20   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

5   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

10   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 of 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

15   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

20   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

5   under soil coarseness (discussed below, section 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

10   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

15   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), especially BG and NAG activities were mostly

constrained by soil TN as suggested by multiple regression models (Table 1). These

20   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

5   specific surface area where microbial cells could attach to 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

10   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,

15   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

20   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

5   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,

10   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

15   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

20   (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 also suggested that soil microorganisms

5   were capable of accumulating P in biomass even under P-depleted conditions (Chapin

et al., 2002; Paul, 2014). Thus, the third hypothesis was supported by our data.

In this study, significant 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

10   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

15   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

20   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 implicate 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 and 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

5   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.

Author contribution

10   Professor X. Han designed the experiment; and L. Lü did the field work to maintain the experiment. H. Liu and X. Feng helped with the measurements of soil analyses. R. Wang wrote the manuscript. C. Creamer and F.A. Dijkstra helped to improve the manuscript. The study was financially supported by the projects from G. Yu and Y. Jiang.

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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

When significant ($P < 0.05$), R values of linear and multiple regressions are shown.

5 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.

Table 2 Relationships between soil pH and soil organic carbon (SOC), total nitrogen

10 (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**

Figures
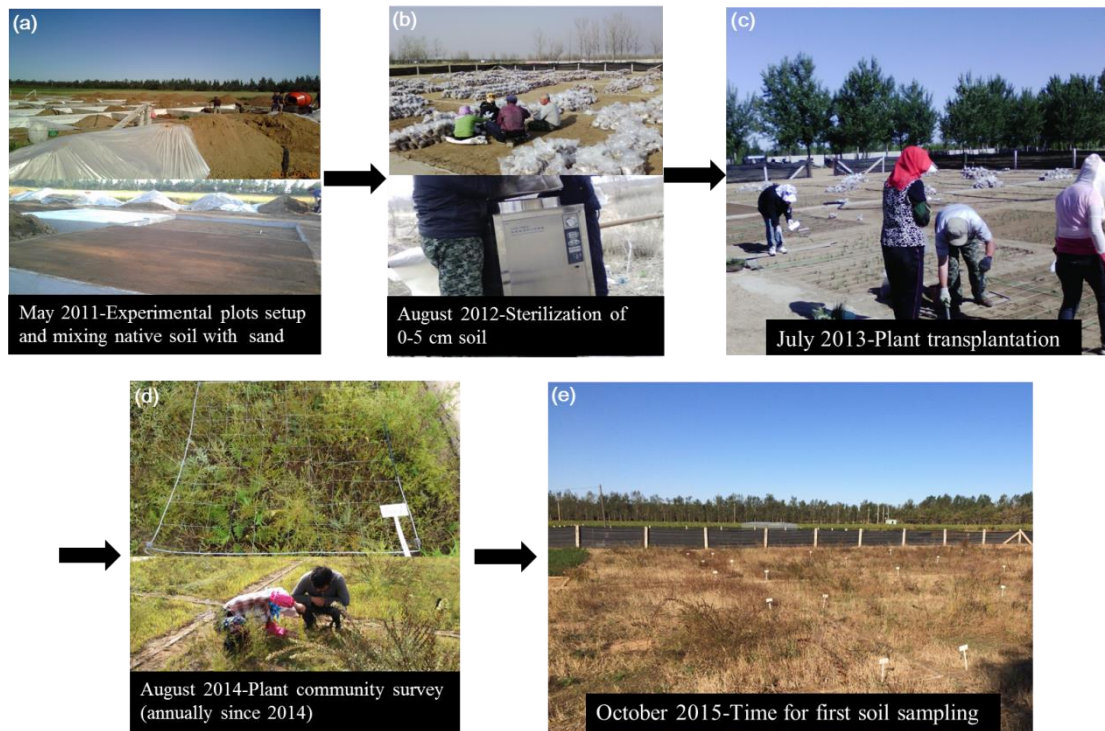
Fig. 1 Overview of field experimentation with photos taken at various stages of the

experiment.
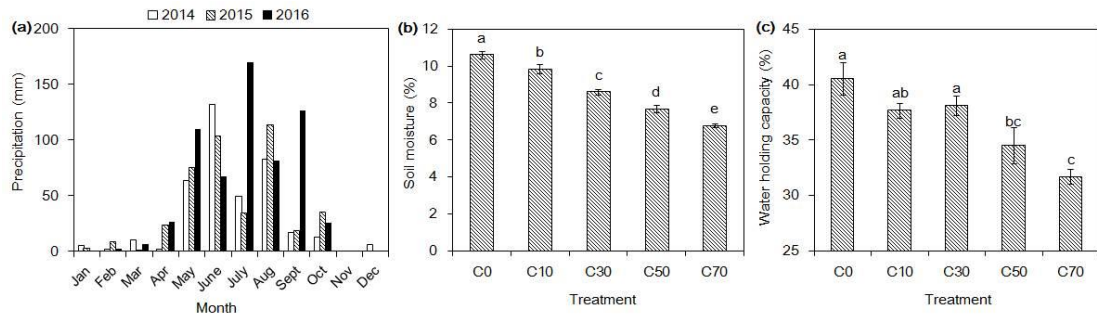
Fig. 2 Monthly precipitation from 2014 to 2016 (a) and response of moisture (b) and water holding capacity (c) in soil samples taken in October 2015 to different degrees of soil coarseness: 0% sand addition (C0), 10% (C10), 30% (C30), 50% (C50), and 70% (C70). Data represent mean ± standard error (n=6). Letters indicate significant differences among treatments.
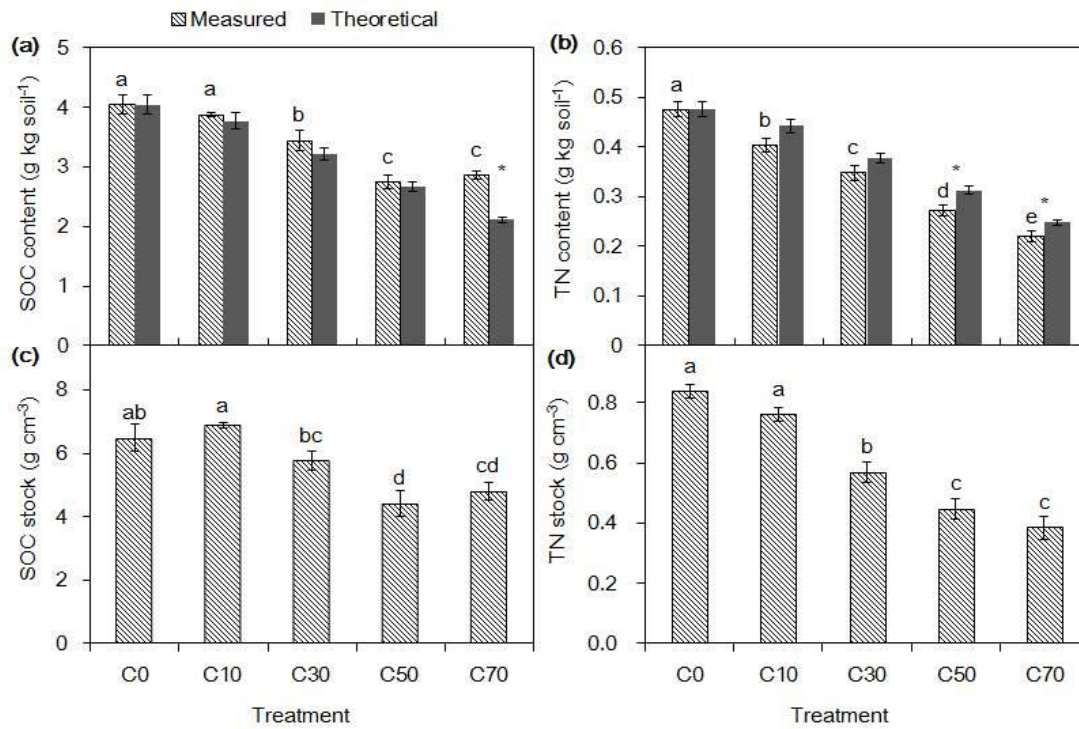
Fig. 3 Soil organic carbon (SOC) and total nitrogen (TN) concentrations (a and b, respectively) and stocks (c and d, respectively) as affected by different degrees of soil coarseness: 0% sand addition (C0), 10% (C10), 30% (C30), 50% (C50), and 70% (C70). Dashed bars represent values obtained from laboratory 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.
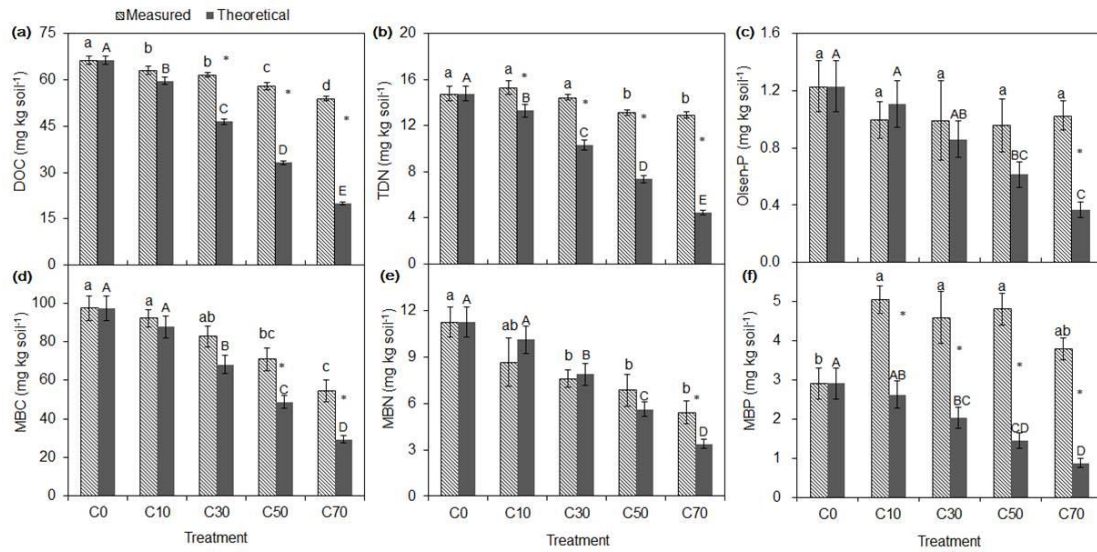
Fig. 4 Changes in soil (a) dissolved organic carbon (DOC), (b) total dissolved nitrogen (TDN), (c) Olsen phosphorus (Olsen-P), (d) microbial biomass carbon (MBC), (e) microbial biomass nitrogen (MBN), and (f) microbial biomass phosphorus (MBP) as affected by different degrees of soil coarseness: 0% sand 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.
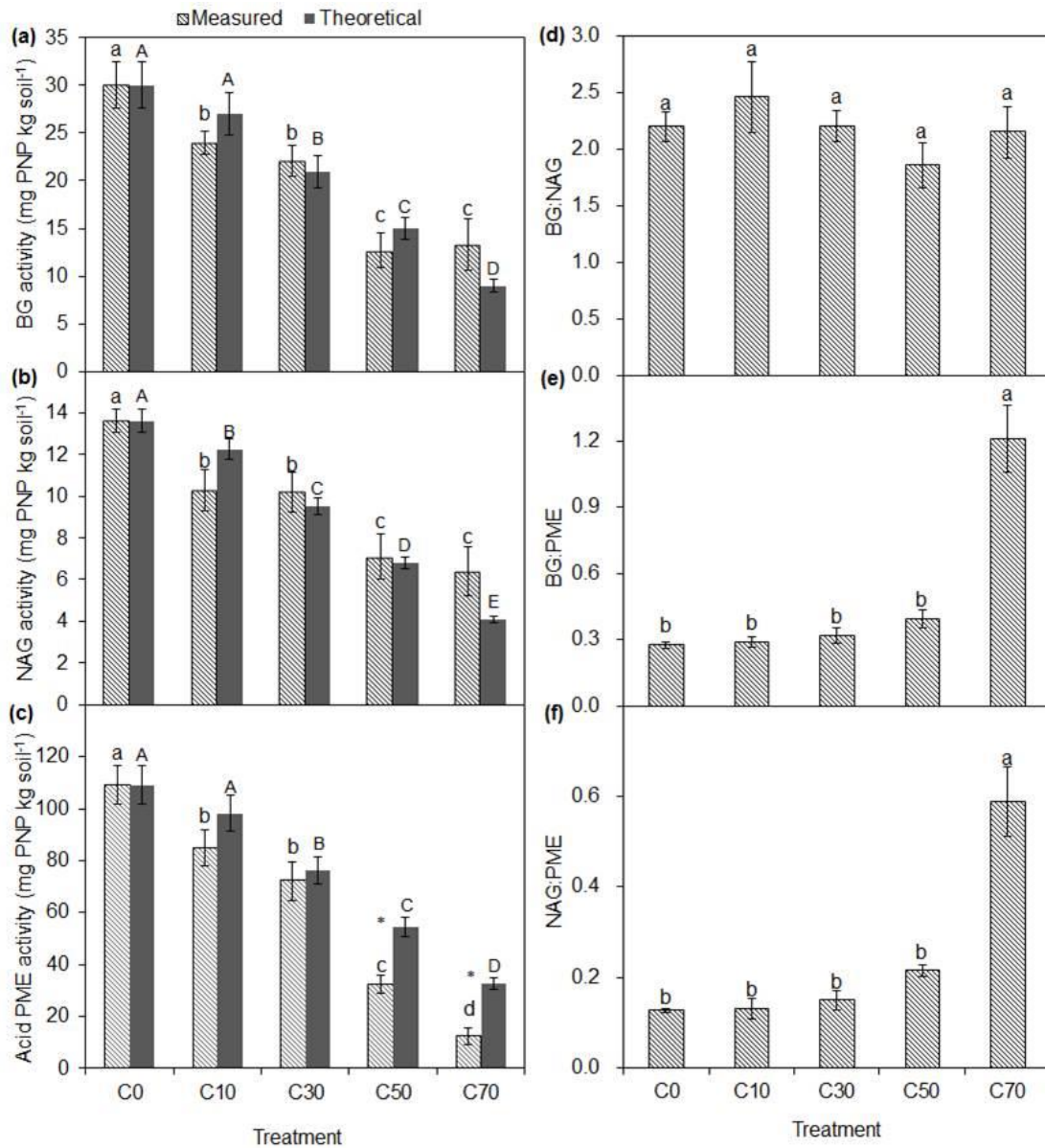
Fig. 5 Changes in (a) activities of soil β-glucosidase (BG), (b) N-acetyl-glucosaminidase

(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%

5 (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.