Response of Carbon and Nitrogen Pools of Vegetation, Soil and Microbe to Different Land-use Patterns in Arid and Semi-arid Grasslands

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Abstract

Previous studies have demonstrated positive net primary production effects with in-creased precipitation in semi-arid grasslands of Inner Mongolian. The knowledge of the store and storage potential of carbon (C) and nitrogen (N) can help us to under-stand how ecosystems would respond to anthropogenic disturbances under different management strategies. Therefore, we carried out research on the storage of organic C and N in four sites where the floras and landform were similar but the intensities of disturbance by grazing animals varied. The primary objective of this study was to pinpoint how the store and storage potential of C and N would respond to grazing exclusion and precipitation. We determined concentrations of both soil organic car-bon (SOC) and soil total nitrogen (TN) in the 0–50 cm soil layers. Concentrations of microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were measured by an innovative method in our study. Additionally, soil bacteria and fungi content were determined in the 0–50 cm soil layers. The total C , N , MBC and MBN storage were significantly different among the four grasslands ($P<0.05$), and they all decreased substantially with grassland degradation and increased to a significant extent with the introduction of natural grassland (ND). More than 90% C and 95% N stored in soil were lost, while they were minor in other pools (including those stored in above-ground biomass, litter, and roots). It is inter-esting to note that micro-aggregate is a limiting factor to soil and microbial nutrients pool compared to precipitation. The limit range of C and N storage observed in these grassland soils suggests that enclosed-fence may be a valuable mechanism of seques-tering C in the top meter of the soil profile. The results of this study can provide a basis for better recovery of grassland that grazing disturbed in semi-arid areas.

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Abstract

Previous studies have demonstrated positive net primary production effects with increased precipitation in semi-arid grasslands of Inner Mongolian. The knowledge of the store and storage potential of carbon (C) and nitrogen (N) can help us better understand how ecosystems would respond to anthropogenic disturbances under different management strategies. Therefore, we carried out research on the storage of organic C and N in four sites where the floras and landform were similar but the intensities of disturbance by grazing animals varied. The primary objective of this study was to pinpoint how the store and storage potential of C and N would respond to grazing exclusion and precipitation. So, the concentrations of soil organic carbon (SOC) and soil total nitrogen (STN) were determined in the 0–50 cm soil layers, and the concentrations of microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were measured by an innovative method in our study. Additionally, the contents of soil bacteria and fungi were determined in the 0–50-cm soil layers.

The research showed that the total C, N, MBC and MBN storage were significantly different among the four grasslands (P <0.05), and they all decreased substantially with grassland degradation and increased to a significant extent with the introduction of natural grassland (NG). More than 90% of C and 95% of N stored in soil were lost, while they were minor in other pools (including those stored in the above-ground biomass, litter, and roots). It is interesting to note that micro-aggregate was a limiting factor to soil and microbial nutrients pool compared to precipitation. The limit range of C and N storage observed in these grassland soils suggested that enclosed-fence might be a valuable mechanism of sequestering C in the top meter of the soil profile. The results of this study can provide a basis for better recovery of the grasslands that grazing disturbed in semi-arid areas.

Keywords : soil organic carbon; microbial activity; semi-arid steppe

1. Introduction

About 40% of the land surface in the world are covered by grasslands (White et al., 2000), most of which are in drylands (Maestre et al.,2012) and sustain the main livestock-production systems (Kemp et al., 2013); Soil contains more carbon than the atmosphere and vegetation combined (Averill C et al., 2014). In some areas, grasslands may serve as important global C sinks. For those in the tropics, the annual sink is about 0.5 Pg of C and it is basically influenced by the baseline of SOC level and annual precipitation (Davidson et al., 1995; Scurlock and Hall, 1998; Tan et al., 2006). Moreover, they store 200–300 Pg of soil carbon, which influences the global carbon cycle significantly (Scurlock and Hall, 1998).

The significant impacts of the differences in land-use and ecosystem management strategies on the storage of C in grasslands has been shown clearly in the past few decades (Lugo and Brown, 1993; Post and Kwon, 2000; Jones and Donnelly, 2004; Billings, 2006; Elmore and Asner, 2006; Liao et al., 2006). Meanwhile, nitrogen is vital for vegetation productivity as well as terrestrial ecosystem stability (Harpole et al., 2007). Due to the close connection of soil C and N cycles, there is substantive concern that the changes in land-use and its integrative effect may alter the storage of C and N in the soil (Houghton et al., 1999). For instance, in the northern China, the severe degradation or desertification of temperate grasslands was just led by rapid livestock expansion (Li, 1994; Dong and Zhang, 2005). The improvement of soil nutrient availability can help the plant recover from a disturbance, promote the fast-growing plants to regrow faster given a improved soil water status, and then facilitate the recovery of plant growth in an ecosystem (Stampfli et al., 2018). Obviously, nitrogen and water availability is of important impacts on the net primary productivity (NPP) of grasslands (Xu et al., 2014), especially those in semi-arid regions where annual precipitation inputs are significantly less than evaporation (Heisler-White et al., 2008). After the drought, N availability could be triggered; meanwhile, there would be improvements in plant absorption, reallocation and N-use efficiency (Mackie et al., 2019). The soil N availability could affect the composition of microbial community and the richness of soil bacteria and fungi.

However, there is a dearth of information regarding the potential of C and N storage due to the absence of stable or mature grassland ecosystems. The knowledge of the store and storage potential of carbon (C) and nitrogen (N) can help us understand how ecosystems would respond to anthropogenic disturbances under different management strategies. Therefore, we conducted a study on organic C and N storage in four sites that were floristically and topographically similar to ascertain the impact of grazing exclusion and precipitation on the store and storage potential of C and N. This study can provide a basis for recovery of the grasslands that grazing disturbed in semi-arid areas.

2. Materials and Methods

2.1 Study Site and Experiment Design

The study site (42°02'27"N, 116deg17'59"E, elevation 1,334 m a.s.l) is located in the south of Duolun County of Inner Mongolia, northern China. The site has a semi-arid climate with mean annual precipitation of 379.4 mm and mean annual temperature of 2.1 degC, ranging from -17.8 degC in January to 18.8 degC in July. The mean annual precipitation from January to June is 118.8 mm and January to August 306.7 mm. The mean annual temperature from January to June is 0.1 degC and January to August 4.69 degC. The soil pH of the experimental site is ranging from 7.1(P =0.04) to 7.4(P =0.003) under precipitation. It is featured by a chestnut soil (Chinese Soil Taxonomy) or Calcisorthic Aridisol (the U.S. Soil Taxonomy). The steppe in that region has been severely degraded due to overgrazing in the past 50 years. In this study, four experimental sites were selected and subjected to SD, MD, LD, and NG respectively (Table 1). Site SD had been exposed to long-term heavy grazing; and an estimated 90% of the above-ground biomass had been consumed by livestock every year. As indicated, the grasslands were severely degraded by the extremely sparse vegetation coverage (<10%). Site MD had also been exposed to long-term heavy grazing, with an estimated 75% of the above-ground biomass consumed by livestock every year. It was moderately degraded with an existing vegetation cover of 10–25%. Site LD had been subjected to long-term free-grazing, and an estimated 65% of the above-ground biomass had been consumed by livestock every year with existing vegetation cover of 25–30%. Influenced by climatic conditions and human activities, the dominant vegetation on aeolian soil was Spiraea saliclfolia and Salix gordejevii. Associated species were Leymus chinensis, Agropyron cristatum, etc. Site NG was set up in 2000 by fencing a 40 ha of a previously free-grazing grassland when the local government initiated a grassland protection program (Xu et al. 2012). Natural grassland (NG) is dominated by needlegrass (Stipa krylovii), wheatgrass (Agropyron cristatum), and prairie sagewort (Artemisia frigida). It is undeniable that there are pseudo-replication issues given that there is only one plot per grazing regime, and in these studies, this problem is quite common. However, it should be certain that changes in SOC and STN among the four plots in this study are mainly caused by grazing intensity and length of exclusion because the four experimental plots are floristically and topographically similar and all are distributed in the same upper basalt platform (Table 1)

2.2 Field Sampling and Laboratory Analysis

In early April 2017, we selected representative plots at site SD, MD, LD, and NG to measure the above-ground and below-ground C and N contents in plants, litter and roots. The field samplings conducted in mid-June and mid-August 2017 in Inner Mongolia were taken as the research objects. In each plot, 5 sampling quadrats (each 1 m * 1 m) were set up at 10-m intervals along a random transect. (The information on vegetation and soil types can be acquired from*http://www.maplet.org* .) The above-ground samples in plants and litter were collected subsequently. Root samples were determined by a soil corer (diameter 7 cm), with 5 sampling points for each site. Similarly, soil sampling was conducted by a soil sampler (diameter, 4 cm), and the soil samples were separately collected from five layers at the depths of 0–10 cm, 10–20 cm, 20–30 cm, 30–40 cm, and 40–50 cm in each sampling point.

2.3 Chemical Analysis

(1) The pH of 0–10 cm soil samples, in H_2O (soil : water 1:5), was tested with a PHS-3S pH meter (Sartorius, Germany).

(2) Soil aggregates were fractionated by a laser particle size analyzer. For particle-size fraction (i.e. into

sand, silt, and clay), 50 g of soil (<2mm) was dispersed in 250 ml of distilled water with a KS-600 probe-type Ultrasonic Cell Disrupter System (Shanghai Precision & Scientific Instrument Co. Ltd., China) set at 360 W; then the different particle-size fractions were detached as per Morra et al.(1991). After isolation, large macro-aggregates (>2000μm), small macro-aggregates (250–2000μm), and micro-aggregates (<250μm) were extracted.

(3) The contents (%) of organic C in the samples of plant, litter, root, and soil were measured by a modified Mebius method separately (Nelson and Sommers, 1982). Then, 0.5 g samples were digested with 5mL SOC solution. The concentration of SOC was determined by chemical oxidation with $K_2Cr_2O_7$solution. Then, approximately 0.2 g air-dried soil was weighed in separate test tubes, with analytical replication for each sample and a total of 5 sub-samples from each treatment. Exactly 10 mL of reaction solution containing 0.032 Mol Ag_2SO_4, 0.06667 Mol $K_2Cr_2O_7$, and 9.39 Mol H_2SO_4 was added to each tube which was then placed in a hot (~200 °C) H_3PO_4 bath for 5 mins. Three tubes containing 0.5 g SiO_2 were also used as blanks. The amount of $K_2Cr_2O_7$consumed by SOC oxidation was determined by titrating the remaining $K_2Cr_2O_7$ in the test tubes after digestion. The SOC oxidation efficiency was determined to be 92%, thus a 1.08 correction factor was used. The total N (%) of plant, litter, root, and soil was measured with the modified Kjeldahl wet digestion procedure (Gallaher et al., 1976) with a 2300 Kjeltec Analyzer Unit (FOSS, Sweden). Then, 0.5 g of air-dried and finely ground soil, in duplicate, were taken for each sample. Afterwards, the soil samples were digested with 18.76 Mol H_2SO_4 for 6 hrs under progressively elevated temperature from 150 degC (1 hr), 270 degC (1 hr) to 380 degC (4 hrs) and then automatically distilled in a Kjeldahl apparatus where the evolved NH_3 was adsorbed by H_3BO_3 (20 g L^{-1}). The yield of NH_3 was titrated by diluted H_2SO_4 (0.02 Mol) and converted into the total amount of nitrogen in soil.

(4) Soil microbial biomass analysis. Soil microbial biomass C (MBC) and N (MBN) were determined by the fumigation-extraction method (Vance et al. 1987). In this study, an innovative method, a soil microbial fumigation device and an improved fumigation method (ZL 2020 3 0785811.3) were employed to extract microbial biomass. During the process of microbial fumigation, the traditional glass drying dish needs to be sealed with paraffin wax, which is not only poor in sealing, but also difficult to control the temperature of constant temperature culture. Therefore, vacuum pump is required in the process of culture to maintain a certain negative pressure. However, it would be challenging to maintain the same batch of samples under the same negative pressure, thus resulting in greater individual differences in the fumigation of samples. Additionally, the result of removing chloroform from the container is not ideal after fumigation. It could be claimed that the employment of the patented device could definitely resolve the defects of the prior art. Then, 10 g of each soil sample was fumigated with ethanol-free chloroform ($CHCl_3$) for 24 hrs at 25 . Meanwhile, another sub-sample was kept at the same conditions without fumigation. After $CHCl_3$ was fully removed, organic C from fumigated and unfumigated soil samples were extracted with 0.5Mol K_2SO_4, with a soil: extractant ratio of 1:4 (w/v), and shaken at 150 rpm for 1 hr. Then, extractable organic C in soil extracts was analyzed by a TOC analyzer (High TOC, Elementar) after the filtration with Whatman no. 2v filter paper. The microbial biomass C and N were measured as the difference between fumigated and non-fumigated samples and normalized to the weight of the soil fraction.

(5) The PLFAs were extracted, fractionated, and quantified as described by Bossio and Scow (1998). Frozen soil aggregate samples (equivalent to 8 g dry mass of soil) were extracted by a mixture of methanol, chloroform ($CHCl_3$), and phosphate buffer in a volumetric ratio of (2:1:0.8) for 2 hrs. The sediment was extracted for another 30 mins. After the extractions, the supernatants were transferred to a separation funnel and put to rest overnight. After the separation, the $CHCl_3$ layer was obtained and dried under N_2. Through the elution with $CHCl_3$, acetone, and methanol successively by silica-bonded phase columns, the polar lipids were separated from neutral lipids and glucolipids (Supelco Inc., Bellefonte, PA). By a mild alkali methanolysis, polar lipids were converted into fatty acid methyl esters. The extractants were then redissolved in 300 μL hexane which contained methyl nonadecanoate fatty acid as an internal standard. Samples were analyzed by an Agilent 6850 gas chromatograph coupled with a flame ionization detector and a HP-5 capillary column (25.0 m × 200 μm × 0.33 μm). Peaks were identified with a microbial identification system (Microbial ID. Inc., Newark, DE, USA). In this study, fatty acids with percentages higher than 0.5% of the total

were considered. The i14:0, i15:0, a15:0, i16:0, i17:0, 14:1O5c, 16:1O7c, cy17:0, 17:1O6c, 17:1O8c, 18:1O7c, cy19:0, 16:1 2OH and a17:0 were used as biomarkers for Germ, while the 18:1O9c, 18:2O6c, 18:3O6, 16:1O5, 10me16:0, 10me17:0 and 10me18:0PLFAs were used as biomarkers for fungi (Zak et al. 1996; Pinkart et al. 2002; Zhang et al. 2015; DeForest et al).

2.4 Statistical Analysis

All data were expressed as mean±1 standard error of mean(SEM). The data for the 0–50-cm soil layer were used to analyze the C and N storage potentials of the grassland. An analysis of variance (ANOVA) was used to assess the effect of land-use change on C and N storage and microbiological differences. All statistical analysis were performed with the software program R(3.4.1). The meteorological data of this study are sourced from China Meteorological Data Network (http://www.worldclim.org). Sigmplot and R software were used for mapping.

3. Results

3.1 Variate of Carbon and Nitrogen Pools

3.1.1 Soil Carbon and Nitrogen Pools

The values of the total soil C storage differed significantly among the four sites ($P < 0.01$), varying from 0.4 g C kg^{-1} for plot SD to 17.5 g C kg^{-1}for plot NG. Similarly, the values of the total N storage differed markedly among the four sites ($P < 0.01$), varying from 0.03 g N kg^{-1} for plot MD to 1.7 g N kg^{-1} for plot NG (Fig. 1). The total C storage decreased substantially with grassland degradation, and increased to a significant extent with the introduction of NG. The C concentration in soil was far higher in the 0–10-cm, 10–20-cm and 20–30-cm soil layers than in other soil layers (Fig. 1a). The N concentration in soil was far higher in the 0–10-cm and 10–20-cm soil layers than in other soil layers (Fig. 1b).

Compared to SD, NG increased the total C and N storage in the 0–50-cm soil layer by 97.3% and 98.1%, with an annual increase rate of 1.71% and 1.72%, respectively. The total C and N storage increased logarithmically with the duration of NG ($P < 0.05$) (Fig.1).

3.1.2 Vegetation Carbon and Nitrogen Pools

The C and N stored in the above-ground biomass were less than 537.2 g C kg^{-1} and 20.1 g N kg^{-1}, respectively (Fig. 2), the C and N stored in the roots were less than 503.5 g C kg^{-1} and 16.5 g N kg^{-1}, respectively., and the C and N stored in the litter were less than 510.0 g C kg^{-1} and 16.6 g N kg^{-1}, respectively, accounting for negligible amounts (<1% of the total) of total C and N storage in the ecosystem. The total C storage (including C stored in above-ground biomass, litter, roots, and 0–50-cm soil layers) differed significantly among the four sites ($P<0.01$), and the C storage varied remarkably among the different pools (Fig. 1 and Fig.2). The amount of C stored in plants accounted for over 90% of the total C storage, the C stored in soil was very low (<10%), compared to other pools, and the amount of C stored in the roots varied from 8.5 g C kg^{-1} for plot SD to 432.1 g C kg^{-1} for plot NG. Similarly, the total N storage (including N stored in above-ground biomass, litter, and roots) differed significantly among different grasslands ($P< 0.01$). The total N storage varied from 8.5 g N kg^{-1} for plot SD to 12.5 g N kg^{-1}for plot NG (Fig. 2).

The C and N storage varied remarkably among the different pools at different season, increase or decrease among the different pools (Fig. 2a and Fig.2b).

The N stored in litter and roots was very low, compared to that in above-ground biomass (Fig. 2).

3.1.3 Microbial Biomass Carbon and Nitrogen Pools

The values of MBC storage differed significantly among the four sites ($P < 0.01$), varying from 0.9 mg MBC kg^{-1} for plot MD to 200.7 mg MBC kg^{-1} for plot NG (Fig. 3a). Similarly, the values of MBN storage differed significantly among the four sites ($P < 0.01$), varying from 0.8 mg N kg^{-1}for plot MD to 32.0 mg N kg^{-1} for plot NG (Fig. 3b). The C stored in the MD were less than 26.9 mg MBC kg^{-1}and 4.41 g MBN kg^{-1}, respectively (Fig. 3a). The MBC and MBN stored in the dry-season were less than 19.6 mg MBC kg^{-1} and

6.25 mg MBN kg^{-1} among the MD, respectively, while the MBC and MBN stored in the wet-season were less than 26.7 mg MBC kg^{-1} and 2.2 mg MBN kg^{-1}among the MD, respectively. The total MBC storage (including MBC stored in the 0–50-cm soil layers) differed significantly among the four sites ($P < 0.01$). The total MBC storage decreased substantially with grassland degradation, and increased to a significant extent with the introduction of NG (Fig. 3).

The amount of MBC stored in NG accounted for over 70% of the total MBC storage in the site, and the MBC stored in deep-soil layer was very low (< 20%), compared to other layers. The amount of MBN stored in the soil varied from 0.5 g MBN mg kg^{-1} for plot MD to36.5 mg MBN kg^{-1} for plot NG.

The MBN concentration in soil was far higher in the 0–10-cm, 10–20-cm, and 20–30-cm soil layer than in other soil layers (Fig. 3).

The total MBC and MBN storage varied remarkably among the different seasons ($P < 0.01$), increase or decrease among the different pools (Fig. 3a and Fig.3b).

Compared to NG, more than 90% of the soil microbial biomass in MD had been lost. Compared to MD, NG increased the total MBC and MBN storage in the 0–50-cm soil layer by 93.5% and 90.7%, respectively.

The total MBC and MBN storage did show a similar trend of increase with the duration of NG (P <0.05) (Fig. 3).

3.1.4 Bacteria and Fungi Pools

The content of sand and gravel contributed more to soil fungi and had a positive effect (Fig. 8). Microorganisms were not always limited by soil C and N, and the bacteria and fungi storage did not show a similar trend of increase with the duration of NG ($P < 0.05$). The percentage of bacteria and fungi varied remarkably among the different grazing pools (Fig. 4). The variation range of soil bacteria percentage in dry-season was 26.5%˜40.7% and wet-season 5.4%˜47.0%, respectively, while the variation range of soil fungi percentage in dry-season was 3.9%˜10.42% and wet-season 1.3%˜8.9%, respectively.

In different desertification, the overall trend of soil fungi is that SD has the lowest level, and MD and LD are similar (Fig. 4).

Our results partially supported a decreased contribution of fungi PLFAs in wet-season compared to dry-season (in the 0-40-cm soil layers) and an increased contribution of fungi PLFAs in wet-season compared to dry-season (in the 40-50-cm soil layers) (Fig.4a and Fig.4b). With the increase of precipitation, soil bacteria and fungi significantly increased or decreased, which indicated that soil microorganism was greatly influenced by precipitation patterns. Bacteria and fungi content are found both highest in dry-season among MD and LD, while the highest content of bacteria and fungi may be distributed in wet-season among NG. Additionally, they are remarkably different from lowest to highest content, SD< MD< LD< NG, in wet-season. The distribution of soil bacteria in the surface layer was significantly higher than that in the bottom layer (0-30cm) among SD, MD, and LD (Dry-season, Fig.4a).

3.2 Relationship between Nutrient Pools and Grazing Intensity

3.2.1 Effects of Soil Aggregate Size on Grazing Intensity

Compared to SD, NG can increase silt and clay storage in the 0–50-cm soil layer by 90.2% and 90.5% (annual increase rates), respectively. In wet-season, changes in grazing management can lead to annual increases of about 1.3% and 1.4% in silt (R^2=0.97) and clay(R^2=0.88) storage, respectively. The soil silt storage sustained an initial rapid increase with the introduction of NG, followed by a steady phase of silt storage with grazing time (Fig. 6).

3.2.2 Relationship between Soil Nutrient Pools and Grazing Gradient

Compared to SD, NG can increase C and N storage in the 0–50-cm soil layer by 97.3% and 98.1%, and the annual increase rates are both about 1.7% (R^2>0.89; R^2[?]0.98) for C and N, respectively. Moreover, the total soil C content is, to a certain extent, dependent on the type of land-use. The soil C and N storage sustained an initial rapid increase with the introduction of NG (Fig. 6).

3.2.3Relationship between Plant Nutrient Pools and Grazing

In our study, regression analysis indicated that there were less relationship between plant nutrients (including C and N stored in above-ground biomass, litter, and roots, $R^2 < 0.5$; in dry-season and wet-season, respectively) and grazing exclusion (Fig. 5).

3.2.4 Relationship between Microbial Biomass Carbon and Nitrogen Pools and Grazing

In our study, data showed that the soil MBC storage had decreased by 93.5%, 85.6%, and 84.7% for plot SD, MD, and LD (R^2=0.98, dry-season; R^2=0.50, wet-season), respectively, and the soil MBN storage had decreased by 90.7%, 95.2%, and 89.5% (R^2=0.74, dry-season; R^2=0.50, wet-season), respectively (Fig. 6). Therefore, a large amount of MBC and MBN has been lost in the last seven decades across grasslands subjected to long-term heavy grazing. In summary, the degradation of temperate grasslands due to long-term heavy grazing has reversed the sequestration potential and led to MBC and MBN loss by erosion and oxidation, instead of the C and N sequestration that has been desirable in the past seven decades.

3.2.5 Relationship of Soil Fungi and Bacteria with Grazing Gradient

The change in land-use has no significant effects on bacteria and fungi content in the grasslands of northern China. Particularly, low correlation coefficients were observed in dry-season and wet-season (R^2<0.20; R^2<0.65) (Fig. 6). Enhanced diffusion rates of nutrients with water addition may benefit bacteria more than fungi, thereby decreasing the F/B ratio as nutrients are more efficiently transported among the four sites.

3.3 Analysis of Driving Factors of Soil Degradation

Our PCA analysis showed that precipitation did not contribute much more to the potentials of soil carbon and nitrogen storage (including C and N stored in above-ground biomass, litter, and roots). The soil silt and clay content may be considered to make the modest contribution to the soil degradation. The second contribution is considered to be the soil carbon and nitrogen storage. The third is the microbial biomass, and the contribution of vegetative sub-banks is greater than that of climatic conditions (Fig. 7 and Fig. 8).

Regression analysis showed that enclosure had a significant positive effect on soil particulate matter composition (R>0.90;P <0.01). Soil micro-glasses contributed more than 70% to soil nutrient pool (Fig. 6), and soil nutrient pool and microbial pool increased functionally with grazing years (R>0.50; P<0.05) (Fig.6, Fig.7, and Fig.8), indicating that soil has strong dependence on and coexistence with disturbances, and soil quality degradation is a synergistic reaction of grazing disturbance intensity. Fencing is one of the most economical and effective measures for natural vegetation restoration of desertified grasslands.

4. Discussion

4.1 Response of Soil Nutrient Pools to Grazing Gradient

The far-ranging employment of free-grazing as a land-use practice is common in the temperate grasslands of northern China. A long period of overgrazing has incurred the decline of grassland productivity, deterioration of grassland, and the soil loss in vast areas (Dong and Zhang, 2005). In the semi-arid grassland, the potentials of C and N storage are approximately 17.5 g C kg^{-1} and 1.7 g N kg^{-1}, respectively. The productivity of grasslands subjected to NG were stable or mature (Xiao et al., 1996; Bai et al., 2004). Moreover, the result from a 17-year study (2000-2017) on MD suggested that the semi-arid grasslands after more than 15 years of grazing exclusion were very weak C source. It had been noticed that after a long period of exclosure (i.e. >15 years), the C and N storage was relatively higher. Seasonal dynamic of C and N storage is not significantly different (P > 0.05). The same phenomenon is observed in our study. One plausible albeit theoretical account of it is that an increase in ANPP would drive greater competition for all resources, some of which are nutrients and water, and such an increased demand on nutrients would drive greater gross rates of soil organic matter (Fig.1). In our observation, total C and N were increased in wet-season (including above-ground biomass, ground litter, and roots). This, similarly, would likely result in more soil organic matter mineralization under natural disturbances, including large-animal grazing. Through altering soil-water content, nutrient availability and heterogeneity, productivity condition, and so forth, grazing exerts its influences on the dynamics of C and N in a grassland ecosystem (Hulbert, 1988; David et al., 1991; Collins and Smith, 2006; MacNeil et al., 2008).

Despite these caveats, the estimation of the potential storage capacity can help us systematically distinguish the effects of different management strategies on the C and N storage of grasslands in northern China. In this study, the value for site NG was about 537.2 g C kg^{-1}, in alignment with the previous estimate of 10–12 kg C m^2 for the area (Wu et al., 2003), and it was higher than the global mean value of 10.6 kg C m^2(Post et al.,1982). Burke et al.(1995) have illustrated that a 50-year period was enough for the recovery of active SOM and nutrient availability. Thus, we suggest that a MD of at least a 20-year duration would be reasonable for the restoration of the semi-arid grasslands from a state of degradation in productivity, SOC and STN storage to a similar one to natural grassland.

In this study, the soil C and N content in the surface layer is relatively high, which is due to the fact that the surface soil can adsorb newly imported organic matter quickly, thus inhibiting the primal effect and soil organic carbon mineralization. The above findings are similar to those in the early study (White et al., 1996).

4.2 Response of Soil Microbial Pools to Grazing Gradients

From the basic data, we observed that soil nutrient pools and soil microbial biomass pools were similar in increase or decrease based on our correlation analysis. The C and N storage varied remarkably with significant positive correlation in the microbial biomass carbon and nitrogen pools, respectively (Fig. 1, Fig. 3, and Fig. 4). And the PCA analysis indicated that silt and clay were more important contributions to the soil microbial biomass C and N storage than soil nutrient pools. Contrary to the study of predecessors, we observed that higher water availability did not play a positive role in soil nutrient pools and micro organisms.

With regard to MBC and MBN storage, the grasslands with a higher potential for MBC and MBN sequestration are those that have been depleted by poor management strategies in the past. Based on our findings, we conclude that the temperate grasslands of northern China exhibit tremendous potential for the increase of their MBC and MBN storage.

4.3 Effects of Precipitation on Carbon and Nitrogen Pool Cycling

This study showed minor effects of precipitation changes on the recovery of soil nutrients and their microbial biomass content (Fig. 1, Fig. 3, and Fig. 8). There is no significant effect with different precipitation patterns (Ma et al., 2020), which may be led by a high recovery, often with a low productivity at low N.

In grasslands with nutrient limits on productivity, the resistance of a natural system to a disturbance decreased, whereas its recovery/resilience increased with growing limited resource levels such as N and P (De Angelis et al., 1989), which again indicated that given limited nutrient resources, ecosystem resistance and recovery/resilience could be inversely related (Herbert et al., 1999).

Our PCA analysis revealed that precipitation did not contribute much more to soil carbon and nitrogen storage potential (including C and N stored in above-ground biomass, litter, and roots) (Fig. 8). Therefore, contrasting with resistance, the recovery of ANPP or plant growth after a dry year or a drought event was often found higher when the precipitation decreased, which was consistent with other previous studies (Fig. 2 and Fig. 6) (e.g., Xu et al., 2009). Among various ecosystems, the more severe and prolonged droughts are, the more time the ecosystems need to recover (Schwalm et al., 2017). However, in the SD, MD and LD experiment, a great increase in the recovery of nutrient with dry-season regimes may be partly attributable to the fact that the vegetation has not been permanently damaged, leaving the vegetation with the potential to survive even under pre-drought conditions (Schwalm et al., 2017). Additionally, it might be ascribed to the compensatory growth and soil nutrients' releases following rewetting (Mackie et al., 2019). Compensatory growth may play a critical role in the rapid recovery (Chen et al., 2020). As recently reported by Sankaran (2019), in these arid and semi-arid savannas, the ability of the plant community to recover from drought stress hinges on the length, severity and frequency of the pre-drought period. Therefore, whether and how the recovery is constrained by the pre-drought may depend on the severity and duration of the pre-drought (Xu and Zhou, 2007) as well as the growth stage of the plant (Sankaran, 2019). The results of current studies show that there is no strong effect on soil resilience following a change in rainfall regime (Fig. 1 and Fig. 2), which is consistent with the result in a semi-arid grassland (Xu et al., 2014). This may be due to the fact that the whole process of normal precipitation, less precipitation, and rewetting cycle is covered by the resilience metric; and an inverse correlation between resistance and recovery may indicate that a trade-off occurs with resilience.

A previous study indicated that water addition significantly increased fungal abundance in all soil aggregate classes (on average by 37.4%). The composition of bacterial community is more affected by rainfall than that of fungal community (Manzoni et al.2012). In our study, the changes of bacteria and fungi variable by precipitation may be the effectiveness of water in wet-season. Comparatively, the content of sand and gravel contributed more to the growth of soil fungi and had a positive effect (Fig. 6), and bacteria have been found to be better adapted to the environment. Soil acidification is conducive to bacterial reproduction but not to fungal (Pennanen et al. 1998). In this study, the same conclusion can be drawn from the slow decreased change of bacteria with altered rainfall patterns. Conversely, it has also been concluded that fungi increased by as much as 80.8%, which could suggest that there could be better adaption of fungi to lower soil pH than that of bacteria (Wang et al. 2004). With the increase of rainfall in the growing season, the growth rates of bacteria and fungi are different, indicating that water has different effects on different microbial groups, consistent with the results of Zhang et al. In addition, higher water availability could further improve substrate diffusion and nutrient accessibility to soil microorganisms, thereby advancing microbial growth and increasing total PLFA concentration and abundance of individual microbial communities (Dungait et al. 2012; Nielsen and Ball 2015).

We observed that given added water, there was a significant decrease in fungal abundance (Fig. 3b) and F/B ratio, indicating a change in the microbial diversity with enhanced bacterial proliferation over fungi under favorable water conditions (Griffiths et al. 1998). This may be ascribed to the different growth strategies of fungi and bacteria (hyphal growth versus individual cells) (Frey et al. 2004). Fungal hyphae can transport nutrients and resources from one microsite to other sites where nutrients are limiting their growth (Strickland and Rousk 2010). However, increased diffusion rates of nutrients with added water may be more beneficial to bacteria than to fungi, thus decreasing the F/B ratio as nutrients are translocated more efficiently within the three soil fractions (Dungait et al. 2012).

4.4 Response of Plant Nutrient Pools to Grazing Gradient

Recent grassland-related field studies in Inner Mongolia showed that a plant NPP of about 1.5 tons ha^{-1} was limited by both N and water as addition above 5.25-17.5 g N m^2yr^{-1} of background increased NPP by 13%-62% (Bai et al., 2010), whereas water addition increased above- and below-ground NPP by 32.9% and 38.3%, respectively (Xu et al., 2010). However, soil microorganisms are not limited by the same factors that constrain plant systems (Hobbie et al.,2005; Wei et al., 2013). For instance, the study by Wei et al. (2013) showed that there were differences in N saturation levels (threshold levels for N demand) between plants and soil microorganism, emphasizing that microbes could be limited by C or P while plants were limited by N (Treseder, 2008). In addition, under higher N availability in temperate grasslands, both the size and activity of soil microbial biomass were found decreased (Gutknecht et al., 2012; Wei et al., 2013).

Plant nutrients are not limited by soil nutrients and microbial conditions, which may be due to the time and space lag of plant succession compared with soil nutrients. Vegetation replacement and nutrient status change require a long buffer period.

Soil organic carbon content decreased gradually from the NG to the SD, but the plant performance was not synchronous. Soil's response to desertification is more sensitive than vegetation, and the change of plant nutrients has a certain lag in time.

4. 5 Effects of Enclosure on Carbon and Nitrogen Pools

Regression analysis showed that enclosure treatment had a significant positive effect on soil aggregates composition and nitrogen pools ($R>0.90$; $P<0.01$). The soil macro-aggregates contributed more than 70% to soil nutrient storage (Fig. 6). Soil nutrient and soil microbial pools increased functionally with grazing year($R>0.50$;$P<0.05$)(Fig. 6). The recovery of the heavy-grazing area to a stable and healthy natural grassland requires more than 50 years of enclosure management (Fig.6). A large amount of evidence from previous studies suggested that a period of 10 years after enclosure of desertified grassland was a process of soil development from quantitative to qualitative change, and organic matter content reached 2.86% after 17 years of containment, 36.7 times of the initial containment. This study showed that there was a high correlation between soil nutrients and microbial nutrients pools with different grazing levels in the fenced time, and the longer control time is, the more annual nutrients return to the soil storage. And the number of soil surface bacteria in the initial stage of fenced grassland is $43.348*10^4$ soils/g. After 17 years of containment, $975.51\ *10^4$ soils/g were observed. The increase is 40.95 times of the initial containment. For SD and MD grassland, the restoration process may be promoted by reseeding of superior native plant seeds under proper organic fertilizer input and enclosure.

5. Conclusion

Land-use change has significant effects on C and N storage in the grasslands of northern China. The storage of C and N has decreased greatly because of grassland degradation led by long-term heavy grazing. The storage potentials of C and N in the semi-arid grasslands are approximately 537.2 g C kg^{-1} and 16.6 g C kg^{-1}, respectively, so, there is huge potential for the increase of C storage in the temperate grasslands of northern China by improvement of grassland use or management. Micro-aggregates availability was suggested to be the main limiting factor of both NPP and microbial biomass C and N storage in this semi-arid grassland soil. Moreover, it is found that the site with 17 years of fencing has the highest level of soil micro-aggregates. Fencing is the most economical and effective measure for natural restoration of degraded grassland and the restoration of heavily grazed areas to stable and healthy takes at least 50 years.

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Author contribution

Xiuli Gao, Junyao Liu, and Shihai Lv conceived and designed this experiment. Xiuli Gao performed the field experiment and processed the data. Daikui Li and Dewang Wang processed the data. Xiao Guan Put forward the proposal and revise the manuscript. Xiuli Gao analyzed the data and wrote the manuscript.

Conflict of interest

No conflict of interest

Data accessibility statement

All data will be uploaded in a repository once accepted.

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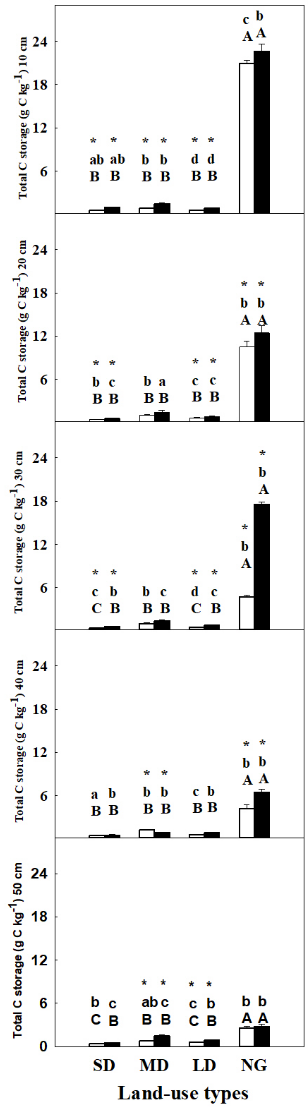
Table 1 Characteristics of experimental plots (DS, dry-season; WS, wet-season)

Land-use types	Location	Dominant and sub-dominant species	pH	Sand (%)	Silt (%)	Clay (%)	Clay (%)	Clay (%)	Clay (%)	Grassland Condition	Grassland Condition	Land-use history	veget cover age DS

SD	116°38´ 43°24´	L.chinensis, S.grandis, C.squarrosa, Artemisia frigida Willd., Salsola collina Pall., Achnatherum sibiricum Keng	7.4±0.1a	98.7±0.3A(WS) 96.0±0.4A(DS)	1.3±0.3C(WS) 4.0±0.4BC(DS)	0.1±0.1B(WS) 0.1±2.8B(DS)	0.1±0.1B(WS) 0.1±2.8B(DS)	Severe degradation	Severe degradation	Severe degradation	Free-grazing, long-term heavy grazing	Free-grazing, long-term heavy grazing	5-15
MD	116°39´ 43°33´	L. chinensis, S. grandis, C. squarrosa, A. frigida, Kochia prostrate Schrad, A. sibiricum	7.3±0.1a	93.5±1.1A(WS) 93.0±0.7A(DS)	6.5±1.1C(WS) 7.0±0.7C(DS)	0.4±0.2B(WS) 4.3±2.3B(DS)	0.4±0.2B(WS) 4.3±2.3B(DS)	Moderate degradation	Moderate degradation	Moderate degradation	Free-grazing, long-term heavy grazing	Free-grazing, long-term heavy grazing	30~3
LD	116°40´ 43°33´	L. chinensis, S. grandis, C. squarrosa, A. sibiricum, A. frigida, S. collina	7.2±0.1a	90.3±2.4 B(WS) 87.6±0.4B(DS)	9.7±2.4B(WS) 12.4±0.4B(DS)	9.7±2.4B(WS) 12.4±0.4B(DS)	0.3±0.1B(WS) 0.1±0.1B(DS)	0.3±0.1B(WS) 0.1±0.1B(DS)	Light degradation	Light degradation	Free-grazing, light grazing	Free-grazing, light grazing	45-50%

NG	116°16´ 42°02	L. chinensis, S. grandis, A. michnoi, A. sibiricum, C. squarrosa, Carex korshinskyi	7.1 ±0.1a	67.3±2.5C (WS) 56.0±1.5C (DS)	30.2±2.4A (WS) 42.1±0.4A (DS)	30.2±2.4A (WS) 42.1±0.4A (DS)	1.9±0.5A (WS) 1.9±0.1A (DS)	1.9±0.5A (WS) 1.9±0.1A (DS)	Natural Grassland	Natural Grassland	40-ha plot that has been fenced since 2000	40-ha plot that has been fenced since 2000	60~6

SD, severe degradation; MD, moderate degradation; LD, light degradation; NG, natural grassland. Values (0–10 cm soil layer) represented as mean±SEM (n=5) and designated by the same letters in the same column are not significantly different at $P < 0.05$.


Total C storage (g C kg-1) 10 cm
Total C storage (g C kg-1) 20 cm
Total C storage (g C kg-1) 30 cm
Total C storage (g C kg-1) 40 cm
Total C storage (g C kg-1) 50 cm
0
6
12
18
24
SD
MD
LD
NG
Land-use types

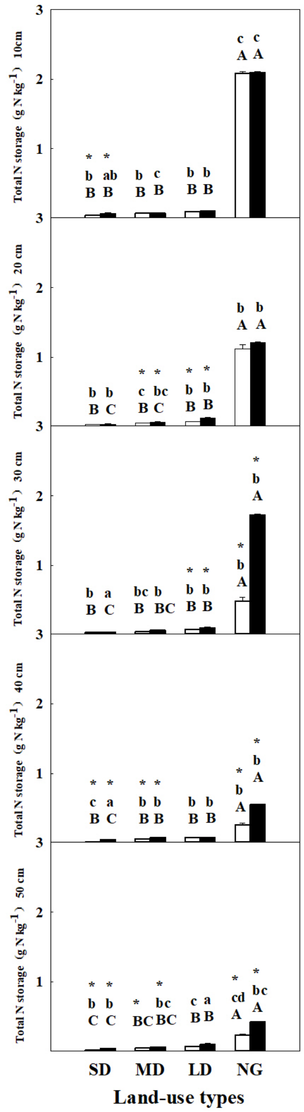
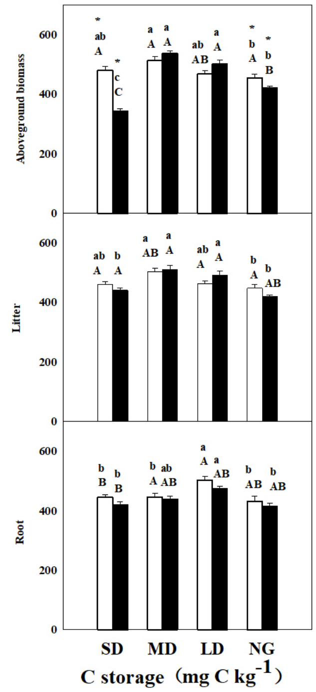


Fig. 1. Changes in total C (1a) storage, and total N storage (1b) based on different land-use types in a semi-arid grassland of northern China (i.e. top 50-cm soil layer). Data are represented as mean± SEM (n = 5). See Table 1 for site abbreviations.


Aboveground biomass
Litter
Root
SD
MD
LD
NG
C storage（mg C kg^{-1}）

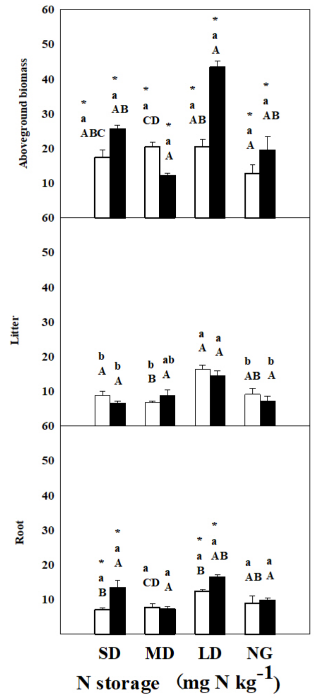
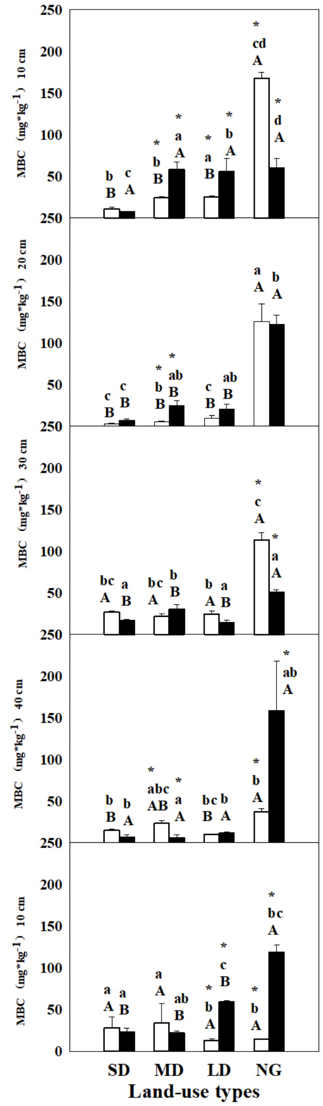


Fig. 2. C and N storage in above-ground biomass (2a), litter (2b), and roots (2c) based on different land-use types in a semi-arid grassland of northern China. Data are represented as mean ± SEM (n = 5). See Table 1 for site abbreviations.



MBC (mg*kg-1) 10 cm
MBC (mg*kg-1) 20 cm
MBC (mg*kg-1) 30 cm
MBC (mg*kg-1) 40 cm
MBC (mg*kg-1) 10 cm
SD
MD
LD
NG
Land-use types

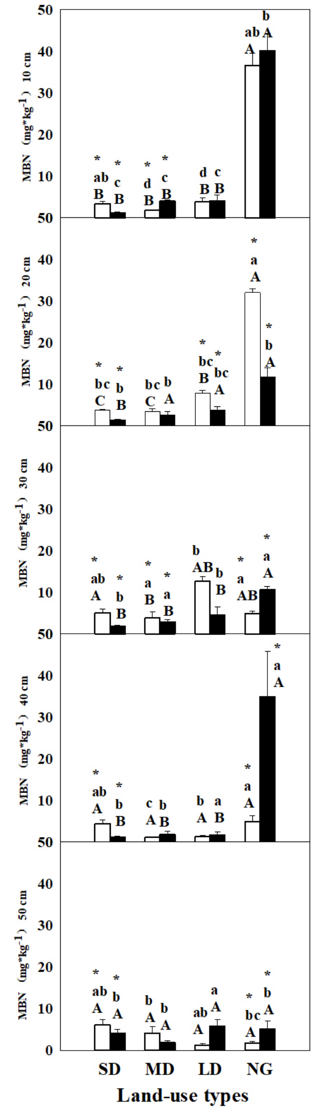
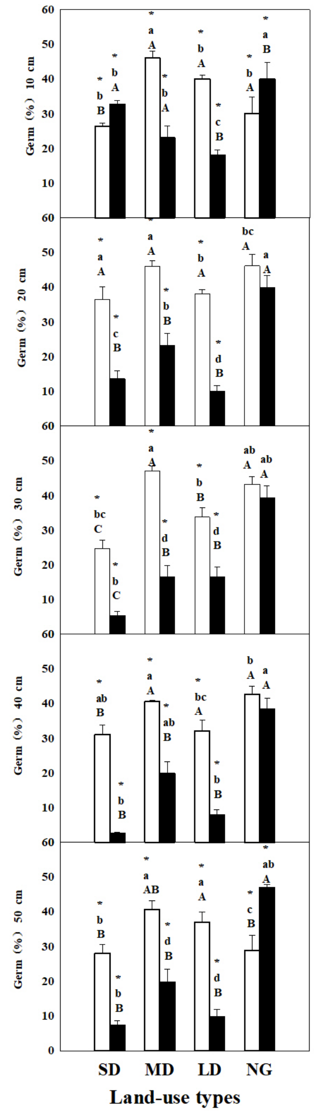


Fig. 3. Microbial biomass C (3a) and N (3b) concentration and distribution based on soil depth in the different land-use types in a semi-arid grassland of northern China. Horizontal bars indicate SEM (n = 5). See Table 1 for site abbreviations.


Germ (%) 10 cm
Germ (%) 20 cm
Germ (%) 30 cm
Germ (%) 40 cm
Germ (%) 50 cm
SD
MD
LD
NG
Land-use types

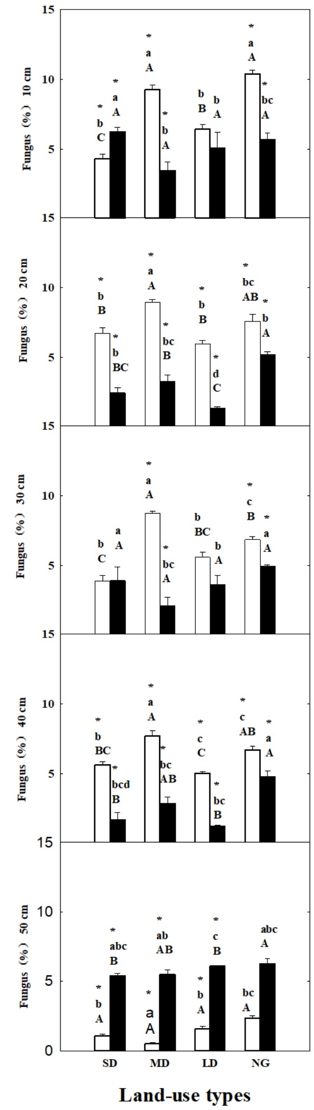
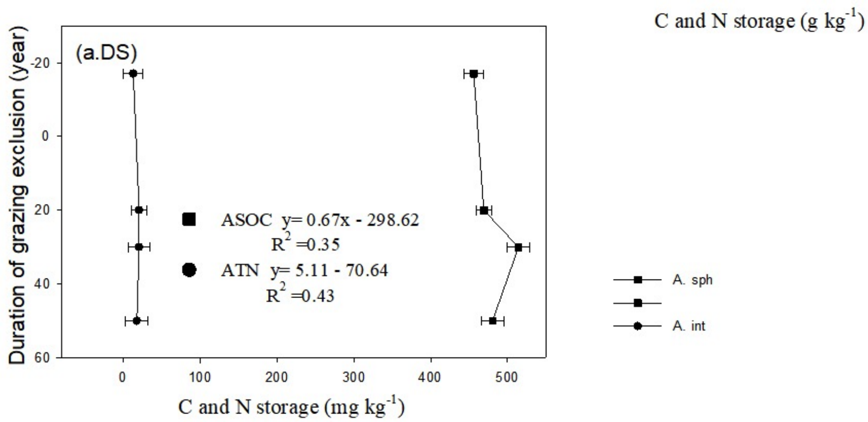
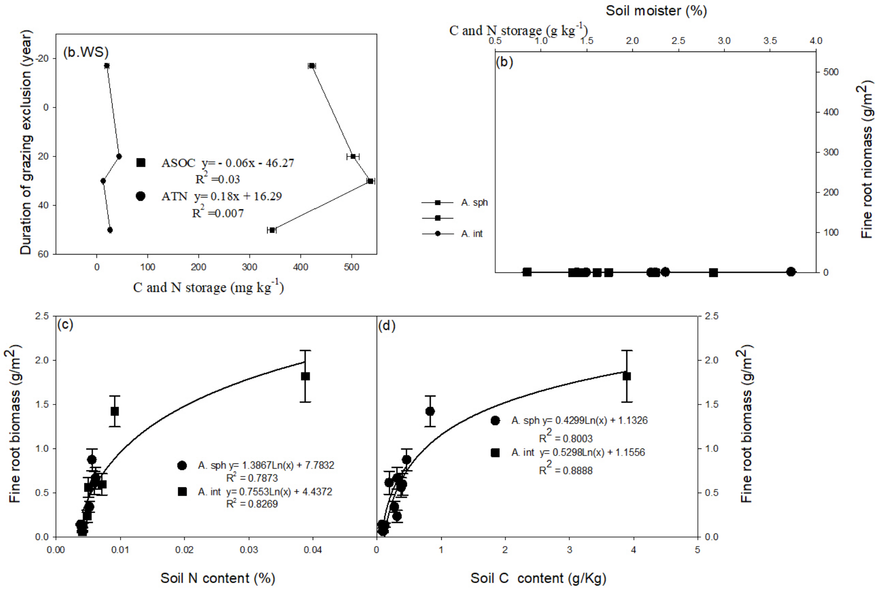
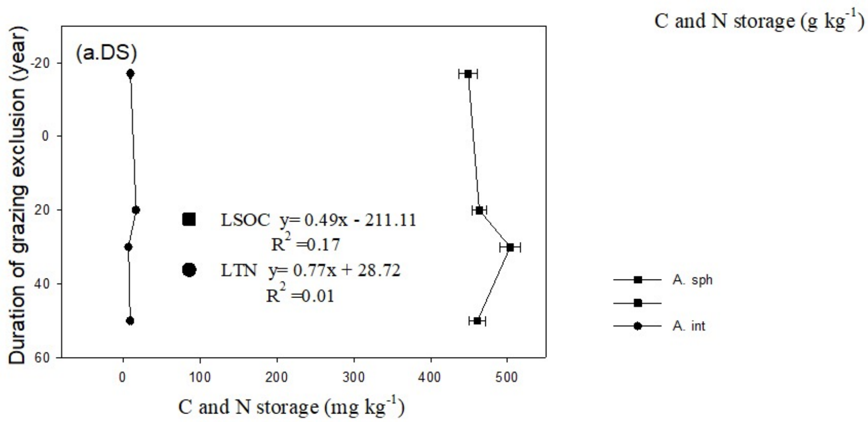
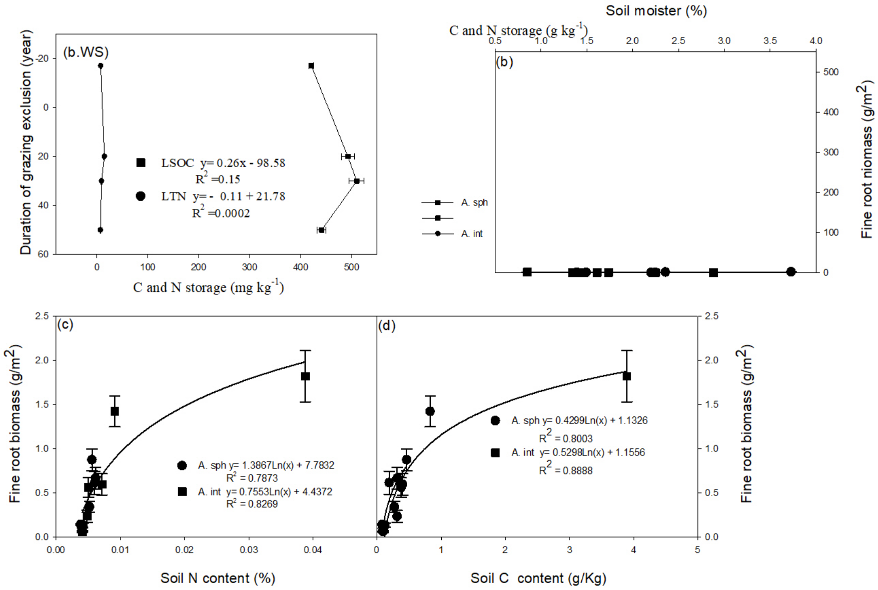


Fig. 4. Percentage of bacteria (4a) and fungi (4b) distribution based on soil depth in the different land-use types in a semi-arid grassland of northern China. Horizontal bars indicate SEM (n = 5). See Table 1 for site abbreviations.

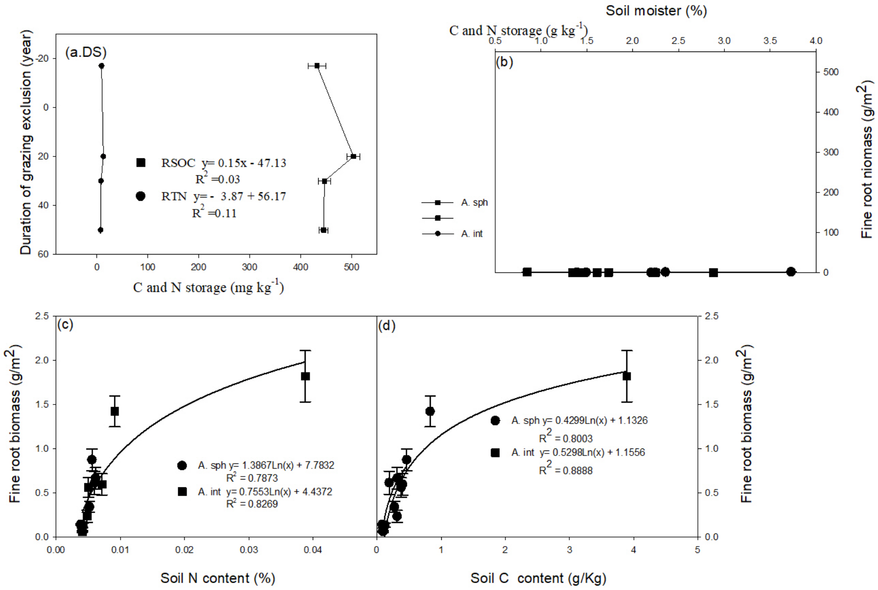

C and N storage (g kg-1)
(a.DS)
Duration of grazing exclusion (year)
ASOC y= 0.67x - 298.62
R2 =0.35
ATN y= 5.11 - 70.64
R2 =0.43
A. sph
A. int
C and N storage (mg kg-1)
-20
0
20
40
60
0
100
200
300
400
500


(b.WS)
Duration of grazing exclusion (year)
ASOC y= - 0.06x - 46.27
R² =0.03
ATN y= 0.18x + 16.29
R² =0.007
C and N storage (mg kg⁻¹)
Soil moister (%)
C and N storage (g kg⁻¹)
(b)
A. sph
A. int
Fine root niomass (g/m²)
(c)
Fine root biomass (g/m²)
A. sph y= 1.3867Ln(x) + 7.7832
R² = 0.7873
A. int y= 0.7553Ln(x) + 4.4372
R² = 0.8269
Soil N content (%)
(d)
A. sph y= 0.4299Ln(x) + 1.1326
R² = 0.8003
A. int y= 0.5298Ln(x) + 1.1556
R² = 0.8888
Soil C content (g/Kg)
Fine root biomass (g/m²)


C and N storage (g kg^{-1})
(a.DS)
Duration of grazing exclusion (year)
LSOC y= 0.49x - 211.11
R^2 =0.17
LTN y= 0.77x + 28.72
R^2 =0.01
C and N storage (mg kg^{-1})
A. sph
A. int
-20
0
20
40
60
0
100
200
300
400
500



(b.WS)
Duration of grazing exclusion (year)
LSOC y= 0.26x - 98.58
R2 =0.15
LTN y= - 0.11 + 21.78
R2 =0.0002
C and N storage (mg kg-1)
Soil moister (%)
C and N storage (g kg-1)
(b)
A. sph
A. int
Fine root niomass (g/m2)
(c)
(d)
Fine root biomass (g/m2)
A. sph y= 1.3867Ln(x) + 7.7832
R2 = 0.7873
A. int y= 0.7553Ln(x) + 4.4372
R2 = 0.8269
A. sph y= 0.4299Ln(x) + 1.1326
R2 = 0.8003
A. int y= 0.5298Ln(x) + 1.1556
R2 = 0.8888
Soil N content (%)
Soil C content (g/Kg)


(a.DS)
Duration of grazing exclusion (year)
RSOC y= 0.15x - 47.13
R2 =0.03
RTN y= - 3.87 + 56.17
R2 =0.11
C and N storage (mg kg-1)
Soil moister (%)
C and N storage (g kg-1)
(b)
A. sph
A. int
Fine root niomass (g/m2)
(c)
A. sph y= 1.3867Ln(x) + 7.7832
R2 = 0.7873
A. int y= 0.7553Ln(x) + 4.4372
R2 = 0.8269
Fine root biomass (g/m2)
Soil N content (%)
(d)
A. sph y= 0.4299Ln(x) + 1.1326
R2 = 0.8003
A. int y= 0.5298Ln(x) + 1.1556
R2 = 0.8888
Soil C content (g/Kg)

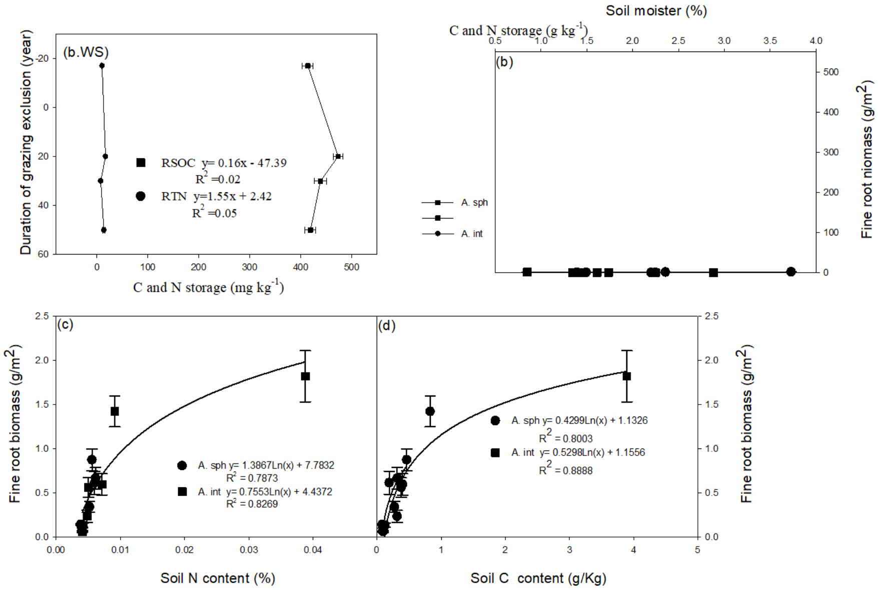
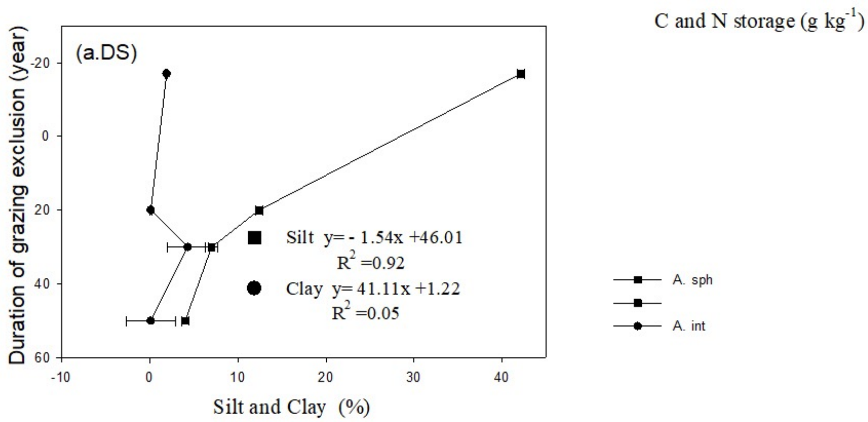
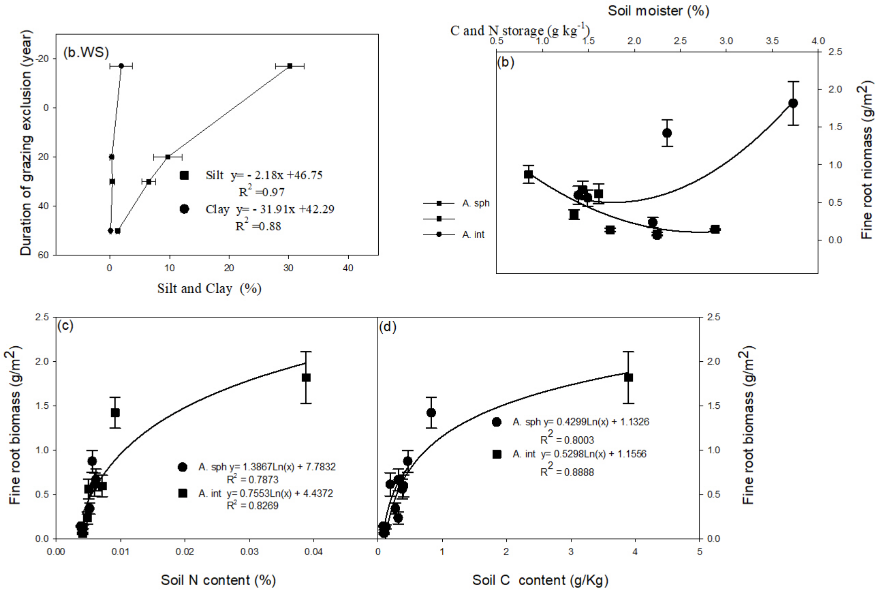
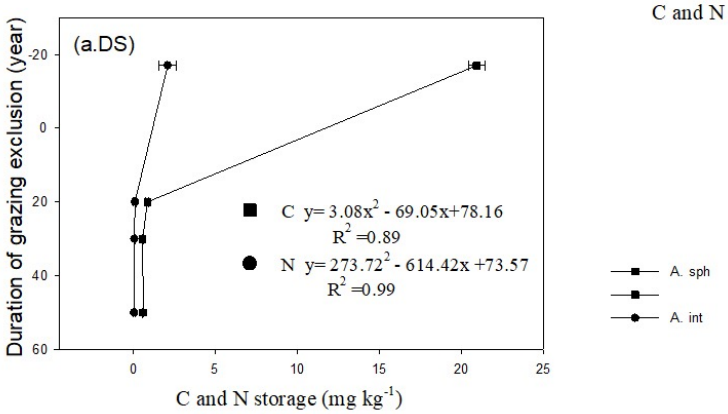


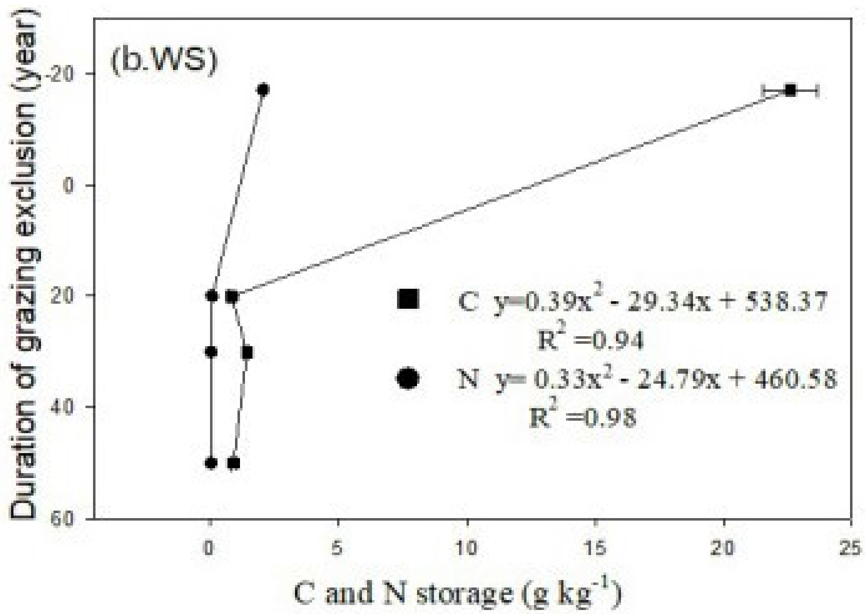
Fig. 5. Relationship between plants nutrient pools and grazing in dry-season (5a) and wet-season (5b). Horizontal bars indicate SEM (n = 5).


C and N storage (g kg-1)
(a.DS)
Silt y= - 1.54x +46.01
R2 =0.92
Clay y= 41.11x +1.22
R2 =0.05
Duration of grazing exclusion (year)
Silt and Clay (%)
A. sph
A. int
-20
0
20
40
60
-10
0
10
20
30
40

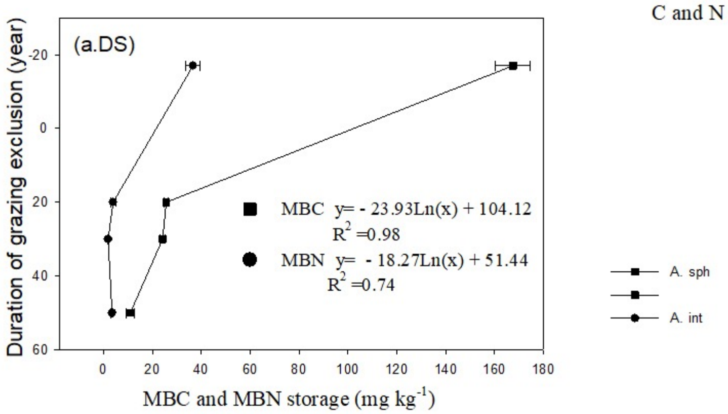

(b.WS)
Duration of grazing exclusion (year)
Silt y= - 2.18x +46.75
R2 =0.97
Clay y= - 31.91x +42.29
R2 =0.88
Silt and Clay (%)
Soil moister (%)
C and N storage (g kg-1)
(b)
A. sph
A. int
Fine root niomass (g/m2)
(c)
Fine root biomass (g/m2)
A. sph y= 1.3867Ln(x) + 7.7832
R2 = 0.7873
A. int y= 0.7553Ln(x) + 4.4372
R2 = 0.8269
Soil N content (%)
(d)
A. sph y= 0.4299Ln(x) + 1.1326
R2 = 0.8003
A. int y= 0.5298Ln(x) + 1.1556
R2 = 0.8888
Soil C content (g/Kg)
Fine root biomass (g/m2)

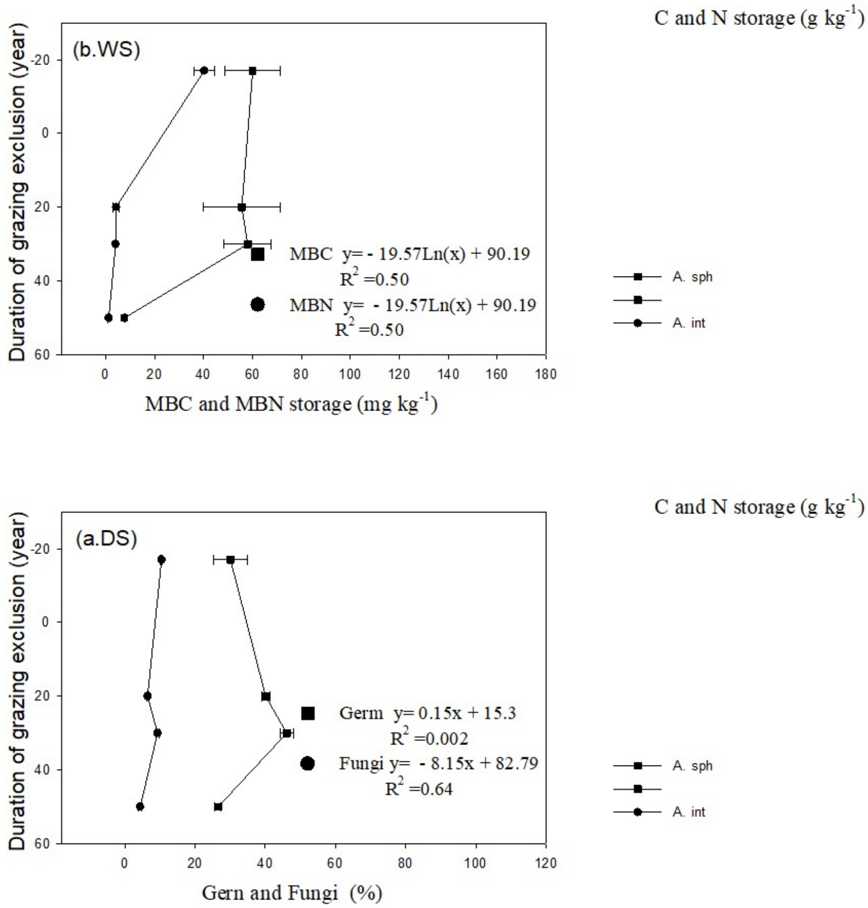
C and N storage (g kg^{-1})





C and N storage (g kg^{-1})




C and N storage (g kg-1)
(b.WS)
Duration of grazing exclusion (year)
MBC y= - 19.57Ln(x) + 90.19
R2 =0.50
MBN y= - 19.57Ln(x) + 90.19
R2 =0.50
MBC and MBN storage (mg kg-1)
A. sph
A. int
C and N storage (g kg-1)
(a.DS)
Duration of grazing exclusion (year)
Germ y= 0.15x + 15.3
R2 =0.002
Fungi y= - 8.15x + 82.79
R2 =0.64
Gern and Fungi (%)
A. sph
A. int

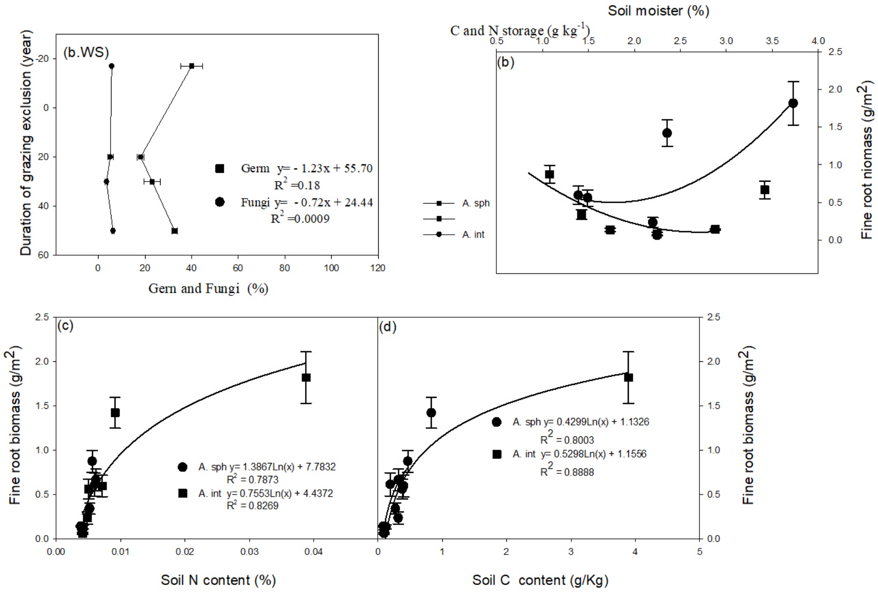


Fig. 6 Changes in soil nutrients pools and microbial pools storage with the duration of grazing exclusion (year) (Dry-season, 6a; Wet-season, 6b). Horizontal bars indicate SEM (n = 5).

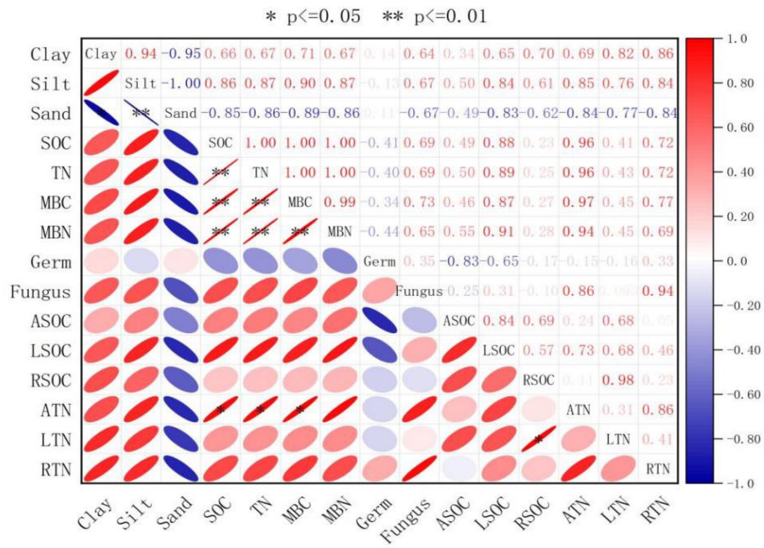


Fig. 7. Correlation Diagram

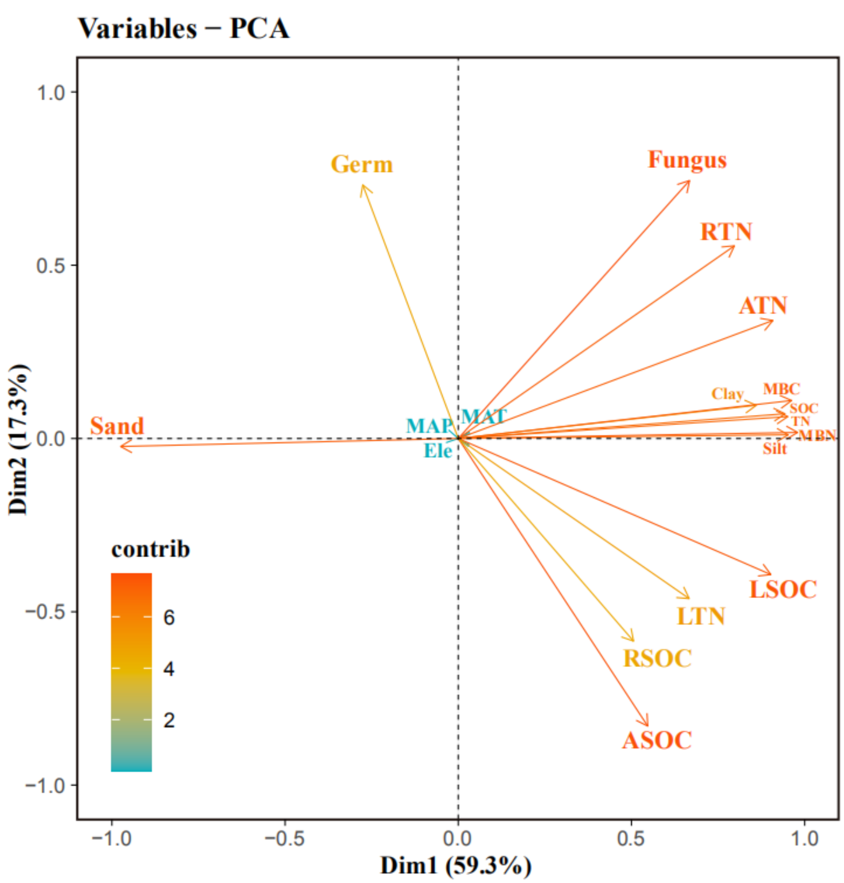


Fig. 8 PCA Analysis