



Effects of mowing and nitrogen addition on the ecosystem C and N pools in a temperate steppe: A case study from northern China

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ABSTRACT

Grasslands, mostly in arid and semiarid regions, play an important role in regulating the dynamics of global terrestrial carbon (C) exchange. However, findings about how global changes affect ecosystem C sequestration are conflicting. To understand how land use and climate change affect ecosystem C and nitrogen (N) pools, this study investigated the effects of mowing and fertilizer on the C and N pools of plants and soil, and on the aggregate-associated C and N content, by means of a field manipulating experiment established in August 2012 in a typical steppe. The results showed that mowing significantly decreased the mass and the C and N pools of shoots by 14.3%, 17.3% and 12.1%, respectively. Compared to ambient N treatments, N addition stimulated shoot biomass and litter mass by 28.8% and 77.4%, respectively, but had no effect on root biomass. Simultaneously, N addition increased the plant N content and the N pools, but it decreased the ratio of plant C to N in the shoots, litter and roots. However, neither mowing nor N addition affected the proportion of macroaggregate, microaggregate, < 0.053 mm size class, geometric mean diameter or aggregate-associated C and N contents. Nitrogen deposition under conditions of climate change stimulated plant productivity but had no effect on soil C sequestration in this short-term experiment. Our results indicate that mowing may be beneficial for providing food for large mammals, but it did not affect soil C and N pools in the study region. Our findings suggest that the response of plant mass and C pools is more sensitive than that of soil C sequestration and soil texture to mowing and N deposition in the temperate steppes of the Mongolian Plateau.

1. Introduction

Global change, resulting from a great increase in the amount of carbon dioxide in the atmosphere, affects the structure and functions of ecosystems (Lin et al., 2010; Deng et al., 2017). Soil contains the largest pool of terrestrial organic carbon (C) and plays a key role in modulating climate change (Li et al., 2013; van Groenigen et al., 2014). Whether the soil C sequestration in land ecosystems can be sustained over the long term depends on the nitrogen (N) dynamics (Deng et al., 2016; Wang et al., 2018). Anthropogenic activities, such as fossil fuel combustion and the application of artificial N-based fertilizers, have increased atmospheric N deposition over the past century (Zhou et al., 2014; Kim et al., 2015). Because of the close coupling of C and N cycles

in soil ecosystems, it is important to understand how N deposition affects ecosystem C sequestration (Lu et al., 2011; Fornara et al., 2013; Heyburn et al., 2017).

However, the effects of N deposition on soil C sequestration are varied. Comprehensive meta-analysis conducted by synthesizing data from multiple terrestrial ecosystems shows that N addition significantly stimulates soil C storage (Yue et al., 2016; Deng et al., 2018), whereas other studies including a 9-year experiment suggest that N fertilizer application has no effect on soil C sequestration (Skinner, 2013). Previous studies have shown that plants tend to develop large root systems to maximize nutrient absorption in natural terrestrial ecosystems (Lebauer and Treseder, 2008; Kong et al., 2017). Nitrogen addition can shift plant resource allocation from belowground to aboveground, thus

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changing the plant C input into the soil (Müller et al., 2000). Moreover, improved N availability is likely to stimulate plant growth and change the quality of plant litter, such as the plant N content and the ratio of plant C to N, and it consequently influences litter decomposition (Mack et al., 2004; Liu and Greaver, 2010; Xia and Wan, 2013; Ziter and MacDougall, 2013; Yang et al., 2018). Further, soil C and N sequestration are closely associated with the stability and structure of soil aggregates (Chaplot and Cooper, 2015; Zou et al., 2018; Chen et al., 2019). According to previous reports, the aggregate size fraction and associated organic C and N are affected by N addition (Wang et al., 2016b; Wei et al., 2016; Zou et al., 2018). The contradictory responses of the soil C pool to N fertilizer application may be caused by varying levels of forage yield, plant litter quality and soil aggregation (Lu et al., 2012; Miao et al., 2019). Interaction between climate change and ecosystem C cycling is likely to be complicated by land use (Luo et al., 2001). Land use change, which is considered the most dominant component of global change, impacts terrestrial ecosystems, profoundly altering land cover, vegetation composition and ecosystem C sequestration (Bahn et al., 2006).

Mowing is a widely practiced grassland management strategy used across the world (Han et al., 2014). Previous studies have shown that mowing may stimulate plant growth through the compensatory growth that allocates proportionally more energy to aboveground biomass and impairs root growth (Crews and Sisk, 2004; Kitchen et al., 2009). Other studies have shown that defoliation decreases plant C inputs due to the decrease of plant biomass (Yang et al., 2012). Root C is the main source of the soil C pool, and the contradictory response of root biomass to mowing may cause variety in soil C pools. Moreover, vegetation removal leads to a rapid decline in the amount of organic matter that is stored in the soil, which in turn suppresses plant growth in semiarid areas (Martinez-Mena et al., 2002; Garcia-Pausas et al., 2011; McSherry and Ritchie, 2013). Thus, the impact of mowing on the soil C pool remains controversial. Further, soil nutrient content controls the effect of land use management on C sequestration by meta analysis (Wang et al., 2011), but the role of nutrient addition in an ecosystem's response to changes in land use is less well understood. Interacting management practices, such as nutrient addition and defoliation, predominantly influence the ecological functions associated with plant tissue and soil. However, the paucity of the interaction effects of mowing and N addition on soil C cycling impedes efforts to establish a clear trajectory of ecosystem C sequestration response to global change in grasslands.

Grasslands constitute upwards of 30% of terrestrial gross primary productivity and produce 10–30% of global soil organic carbon (SOC); thus they play an important role in regulating the dynamics of global terrestrial C exchange (McSherry and Ritchie, 2013; Poulter et al., 2014; Gao et al., 2016; Wang et al., 2016a; Ward et al., 2016; Forrester et al., 2017). The temperate steppe, located in the semiarid and arid regions of northern China, is a typical vegetation type across vast areas of the Eurasian Continent, and this steppe is sensitive to climate change and land use change (Niu et al., 2008; Song et al., 2016; Sagar et al., 2019; Zhong et al., 2019). In addition to nutrient enrichment, hay harvesting, which removes the majority of current-year aboveground biomass, is a widely used practice in this temperate steppe (Liu et al., 2018b). Predicting the response of ecosystem C and N pools to changes in land use and atmospheric composition is challenging, due to the number of influencing factors involved and the possibility of interactive effects across various aspects of grassland ecosystems (Zhu et al., 2016). In this study, we address the following questions: (i) To what extent has plant biomass and C and N pools been altered by mowing and N enrichment? (ii) To what extent has the soil C and N content in bulk soil and aggregates been altered by mowing and N enrichment in a temperate steppe in Northern China?

2. Materials and methods

2.1. Study site and experimental design

The study sites were located in a temperate steppe in the Mongolian Plateau in northern China (42°02'N, 116°17'E, 1324 m). This area has a continental, monsoon climate with an average annual temperature of 2.2 °C and average annual precipitation of 373.7 mm, with 91.3% of this precipitation falling from May to October. The soil texture is sandy loam consisting of 71.9% sand, 15.6% silt and 12.5% clay. The sandy soil is classified as Haplic Calcisol according to the FAO system, and the pH of this soil is 6.8. It is a typical steppe plant community, dominated by *Stipa krylovii*, *Leymus chinensis* and *Artemisia frigida*.

In August 2012, five replicates of each of four groups, control (ambient N addition and no mowing), mowing, N addition and mowing plus N addition, were set up with a random design. Each plot was square with the size of 4 × 4 m², with a 1-m buffer zone between plots. The N enrichment level (10 g N m⁻² yr⁻¹) was within the range of airborne nutrient deposition observed in northern China (Yang et al., 2012). Starting in 2013, NH₄NO₃ was applied to the soil surface in each N addition plot once a year after precipitation in early June. Starting in 2012, aboveground plant parts were removed at the height of 5 cm in the mowing plots once a year in early September when the plants began to yellow to simulate hay harvesting, a widely practiced land use technique in this steppe (Xia and Wan, 2013; Liu et al., 2018b).

2.2. Plant sampling

In late August 2016, when the biomass peaked, one 0.5 × 0.5 m² quadrat was examined in each plot. In each quadrat, all green shoots and the entire litter layer were cut and collected. To measure the root biomass, soil samples were collected at soil depths of 0–40 cm in three places within each quadrat using a 9 cm diameter root auger. The roots collected in the soil samples were isolated by spreading the samples in shallow trays through a 0.5 mm mesh sieve. The shoot parts, litter and root tissues were dried at 65 °C for 48 h and weighed to determine the dry mass. The plant C and N contents was determined using a VARIO EL III CHON analyzer (Elementar, Germany).

2.3. Soil sampling and determination

Soil samples were taken at three points in the soil from 0 to 10 cm and 10–20 cm in the quadrats near the root biomass sampling points, and these samples were then mixed together to make one sample. The soil samples were air-dried and passed through a 0.25 mm sieve to measure the soil C and N content. Soil bulk density was measured at soil depths of 0–10 cm and 10–20 cm in each quadrat using a stainless steel cutting ring that was 5 cm tall by 5 cm in diameter. The soil cores were dried at 105 °C for 24 h. Two representative soil samples of 0–10 cm and 10–20 cm depths were randomly collected from each quadrat to measure the aggregate size distribution. Aggregate size classes were separated by wet sieving through 0.25 and 0.053 mm sieves (Qiu et al., 2015). The macroaggregate (> 0.25 mm), microaggregate (0.25–0.053 mm) and < 0.053 mm size classes were dried in an oven at 50 °C for 24 h and then weighed. After the soil carbonates were removed using 0.2% (vol) hydrochloric acid, the soil organic C and total N content in the bulk soil and the aggregates was analyzed using a VARIO EL III CHON analyzer (Elementar, Germany).

2.4. Data analysis

The mean geometric diameter (MGD) was calculated by the following equation (Qiu et al., 2015):

$$MGD = \exp \left[\left(\sum_{i=1}^n w_i * \ln x_i \right) / \left(\sum_{i=1}^n w_i \right) \right]$$

where w_i is the mass fraction (%) of aggregates in the i th size class to the total mass of the sample, and x_i is the mean diameter of each class (mm).

We used the following equations to calculate the soil organic carbon pool (SCP) and the soil nitrogen pool (SNP) (Wang et al., 2014):

$$SCP = BD \times SOC \times D$$

$$SNP = BD \times TN \times D$$

where SCP is the soil organic carbon pool (kg m^{-2}); SNP is the soil nitrogen pool (kg m^{-2}); BD is the soil bulk density (g cm^{-3}); SOC is the soil organic carbon content (g kg^{-1}); TN is the soil nitrogen content (g kg^{-1}); and D is the thickness of the sampled soil layer (m).

2.5. Statistical analyses

The effects of mowing and N addition on the mass, plant C content, plant N content, ratio of plant C to N, plant C pool and plant N pool of the shoots, litter and roots, and on the mass proportions of grass, forb and legumes were examined by two-way ANOVAS. The effect of soil depth, mowing and N addition on the proportion of the macroaggregates, microaggregates (%), < 0.053 mm size class, mean geometric diameter, C and N content in the bulk soils and aggregates, and the soil C and N pools, were examined by three-way ANOVAS. Mowing and N addition were found to have no significant effect on the soil C and N content of the different aggregate sizes. Therefore, we tested the effect of aggregate size on the C and N content by one-way ANOVAS and the Duncan's post hoc test to establish multiple comparisons. All statistical analyses were performed using the software program SPSS, ver. 16.0 (SPSS Inc., Chicago, IL, USA). Differences at $P < 0.05$ were considered statistically significant.

3. Results

3.1. Plant carbon and nitrogen pool

Mowing significantly decreased the shoot and litter biomass by 14.3 and 46.8%, respectively (all $P < 0.05$, Fig. 1, Table 1). Nitrogen addition significantly stimulated shoot biomass and litter mass by 28.8 and 77.4%, respectively (all $P < 0.05$, Fig. 1, Table 1). Neither mowing nor N addition affected root biomass. Mowing and N addition had a

marginally interactive affect on shoot biomass ($P = 0.1$). Mowing decreased shoot biomass by 31.7 and 65.6 g m^{-2} under ambient nitrogen treatment and nitrogen treatment, respectively. Nitrogen addition stimulated shoot biomass by 96.4 and 62.5 g m^{-2} in the unmown and mowed plots, respectively. Nitrogen addition significantly increased the grass functional group biomass proportions by 18.9%, but decreased the forb functional group biomass by 16.3% (absolute change, all $P < 0.05$, Fig. 1, Table 1).

Nitrogen addition significantly decreased the root C content ($P < 0.05$, Fig. 2, Table 1). Neither mowing nor N addition affected the shoot or litter C content. Nitrogen addition significantly stimulated the plant N content of shoots, litter, and roots by 22.4, 31.0, and 18.7%, respectively. Nitrogen addition decreased the ratio of plant C to N of shoots, litter, and roots (all $P < 0.05$, Fig. 2, Table 1). Mowing did not influence plant N content or the ratio of plant C to N.

Mowing significantly decreased the plant C pool of shoots and litter by 17.3 and 49.1%, respectively (all $P < 0.05$, Fig. 3, Table 1). Nitrogen addition stimulated the plant C pool of shoots and litter by 31.8 and 81.3%, respectively (all $P < 0.05$, Fig. 3, Table 1). Neither mowing nor N addition affected the plant C pool of roots. Mowing significantly suppressed the plant N pool of litter by 49.1% ($P < 0.05$, Fig. 3, Table 1). Nitrogen addition significantly stimulated the plant N pool of shoots, litter and roots by 60.5, 136.3 and 37.1%, respectively (all $P < 0.05$, Fig. 3, Table 1). There was no interactive effect of mowing and N addition on the plant C pool or the plant N pool (Table 1).

3.2. Soil carbon and nitrogen pool

The soil C content, soil N content and ratio of soil C to N varied significantly between the 0–10 cm soil samples and the 10–20 cm soil samples (all $P < 0.05$, Fig. 4, Table 2). Across the two soil depths, neither mowing nor N addition affected the soil C content, the soil N content, the soil C pool or the soil N pool (all $P > 0.05$). The proportion of the three aggregate size classes varied significantly between the 0–10 cm soil samples and the 10–20 cm soil samples (all $P < 0.05$, Fig. 5, Table 2). Mowing and N addition had no effect on the proportion of macroaggregates, microaggregates or the < 0.053 mm size class, or on the mean geometric diameter at both the 0–10 cm and 10–20 cm soil layers (all $P < 0.05$, Fig. 5, Table 2). Further, neither mowing nor N addition affected the soil C and N content of macroaggregates,

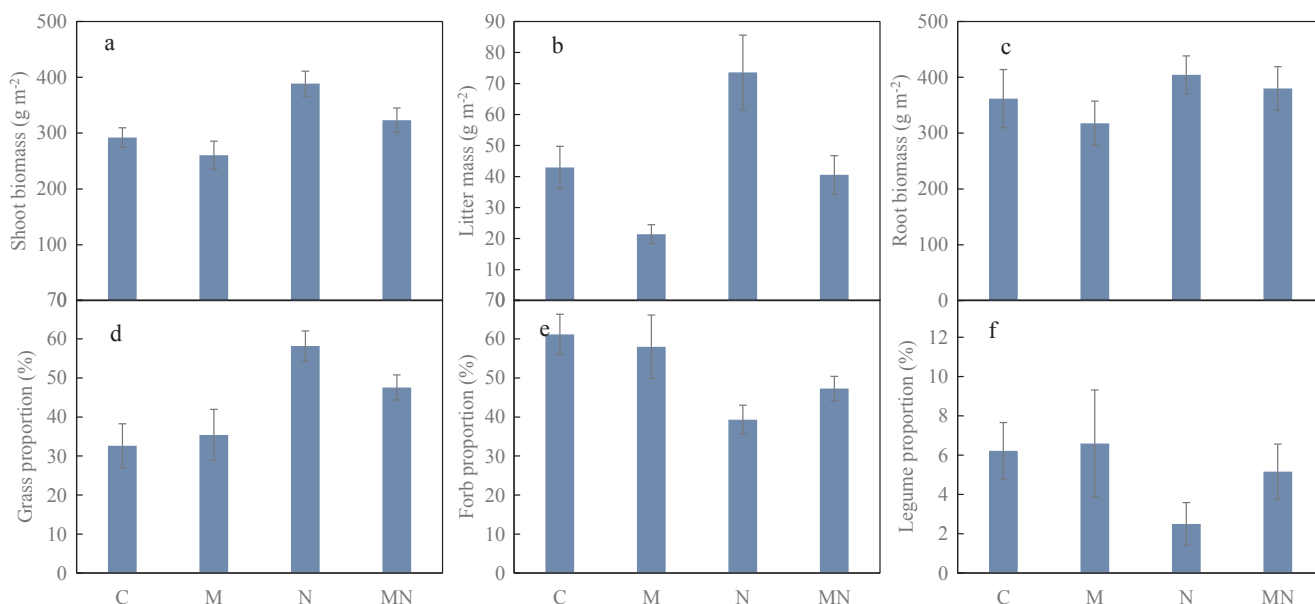


Fig. 1. Shoot biomass (a), litter mass (b), root biomass (c), grass proportion (d), forb proportion (e), and legume proportion (f) under the control (C), mowing (M), nitrogen addition (N), and mowing plus nitrogen addition (MN) treatments. Error bars are the standard errors of the means.

Table 1

The effects of mowing (M) and nitrogen addition (N) on mass, plant carbon content, plant nitrogen content, ratio of plant C to N, plant carbon pool, and plant nitrogen pool of shoot, litter and root, respectively, and the mass proportions of grass, forb, and legume.

F-value	Mass			Functional group proportions			Plant carbon content			Plant nitrogen content		
	Shoot	Litter	Root	Grass	Forb	Legume	Shoot	Litter	Root	Shoot	Litter	Root
M	4.91	12.491	0.67	0.609	0.196	0.727	2.373	0.259	1.224	0.887	2.233	0.257
N	13.116	10.397	1.583	14.192	9.1	2.109	1.336	0.012	5.695	25.225	17.538	7.053
M*N	0.596	0.559	0.054	1.796	1.064	0.414	3.362	0.004	0.109	1.322	0.861	0.194
F-value	Ratio of plant C to N			Plant carbon pool			Plant nitrogen pool					
	Shoot	Litter	Root	Shoot	Litter	Root	Shoot	Litter	Root			
M	2.179	2.215	0.066	7.153	9.988	0.248	2.925	10.573	0.153			
N	14.795	10.133	8.183	15.072	7.862	0.57	37.9	22.812	7.166			
M*N	0.168	1.033	0.052	1.839	0.563	0.024	1.88	2.067	0.3			

Bold values were significant at $P < 0.05$.

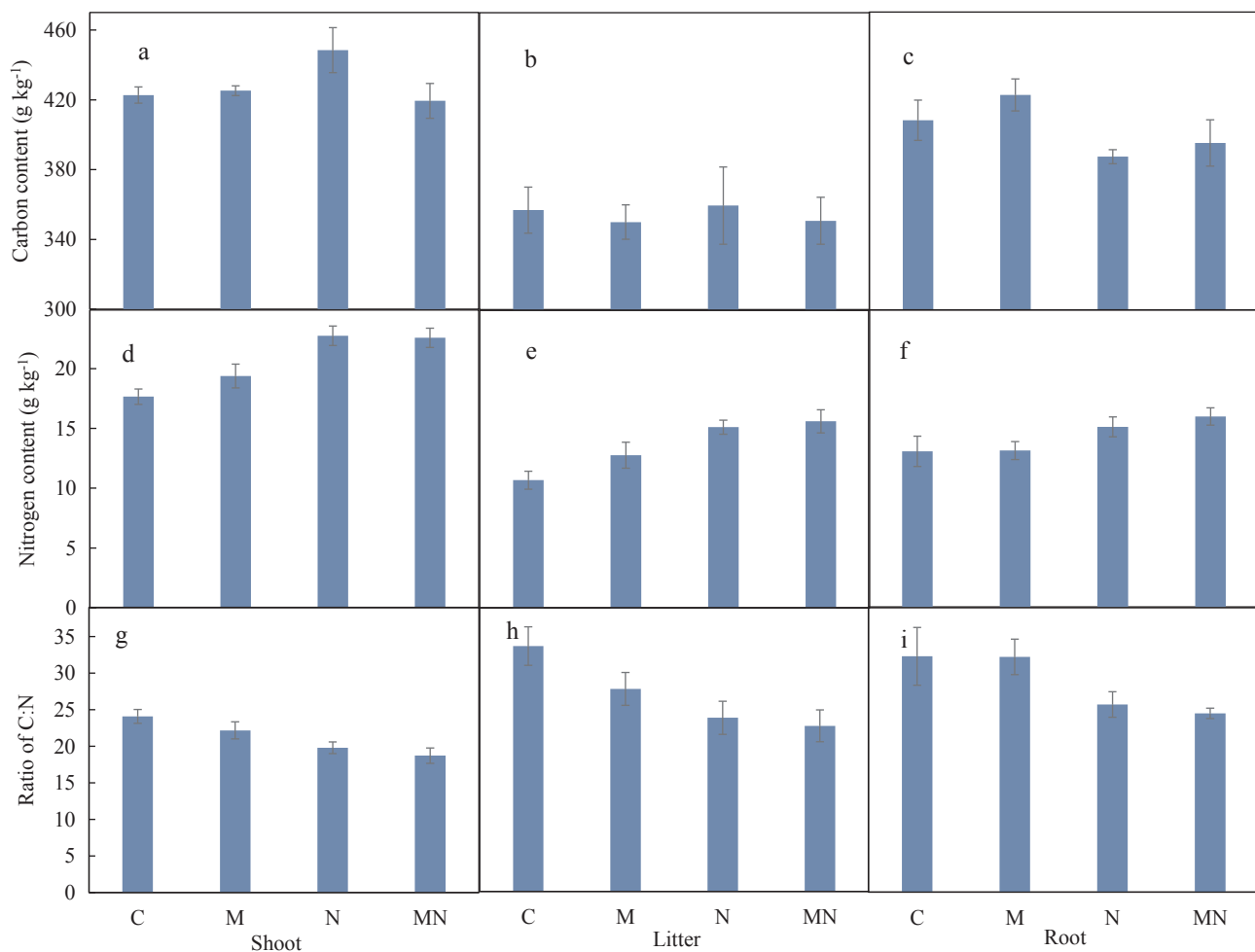


Fig. 2. Plant carbon content, nitrogen content, ratio of C to N in shoot (a, d, g), litter (b, e, h) and root (c, f, i) under the control (C), mowing (M), nitrogen addition (N), and mowing plus nitrogen addition (MN) treatments, respectively. Error bars are the standard errors of the means.

microaggregates or the < 0.053 mm size class at the 0–10 cm and 10–20 cm soil layers (all $P > 0.05$, Fig. 6, Table 2). For all of the mowing and N addition treatments, the soil C content and soil N content was significantly larger in the < 0.053 mm size class than in the macroaggregates and microaggregates (all $P < 0.05$, Fig. 6).

4. Discussion

4.1. The responses of plant biomass and C and N pools to treatment

In our study, N addition stimulated the shoot and litter mass, but it had no effect on the root biomass after a 4-year experiment. Nitrogen, which is often the main limiting element for plant growth in semi-arid grassland ecosystems (Lebauer and Treseder, 2008), is an important constituent of proteins and plays an essential role in plant production,

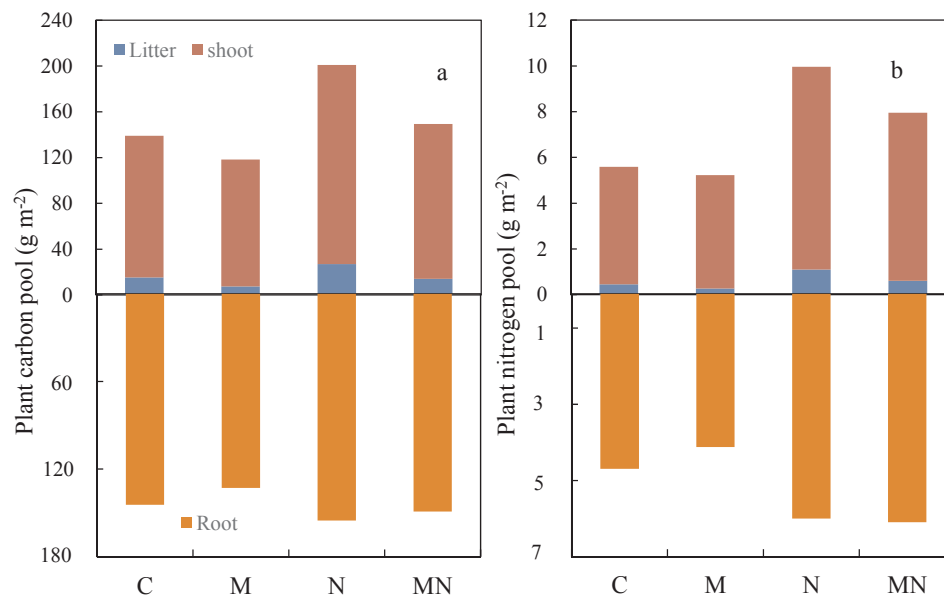


Fig. 3. Plant carbon pool (a), and plant nitrogen pool (b) in shoot, litter and root under the control (C), mowing (M), nitrogen addition (N), and mowing plus nitrogen addition (MN) treatments, respectively.

photosynthesis and litter decomposition (Yang et al., 2018). The stimulating effect of N addition on shoot and litter mass in this typical steppe is similar to that of fertilization stimulation on plant productivity; indeed, N addition was shown to increase nutrient content in a 7-year field experiment in the eastern region of the Eurasian steppe zone (Liu et al., 2018a). Previous studies have shown that an increase in soil nutrient availability shifts plant resource allocation and causes a reduction in total root biomass (Bardgett et al., 1999; Müller et al., 2000; Heyburn et al., 2017). However, the root biomass at soil depths from 0 to 40 cm demonstrated no response to N addition, possibly because of the changes in plant composition after the 4-year treatment in this study. On the one hand, forb is the functional group with the most significant deep-root system (Su et al., 2018), and suppressing N addition in the forb functional group biomass may decrease root

distribution in the deep soil layer. On the other hand, grass is the more resource-acquisitive plant functional group, comprising shallower-rooted species, so the stimulation of N addition on the grass functional group biomass may increase root distribution in the surface soil layer (Stampfli et al., 2018). The response of the plant C and N status to soil N availability is critical for the projection of nutrient cycling dynamics under future global change scenarios (Lu et al., 2012). The stimulation of plant N content and the suppression of the ratio of plant C to N under the N addition treatment both indicate that plant N content is sensitive to climate change in the temperate steppe (Han et al., 2014). The stimulation of N addition on the plant C pools of shoots and litter is caused by the increase in the biomass. Moreover, our results indicate that more N was fixed in the shoots and litter, the greater the stimulation effect on both biomass and plant N content in this ecosystem. The increase of

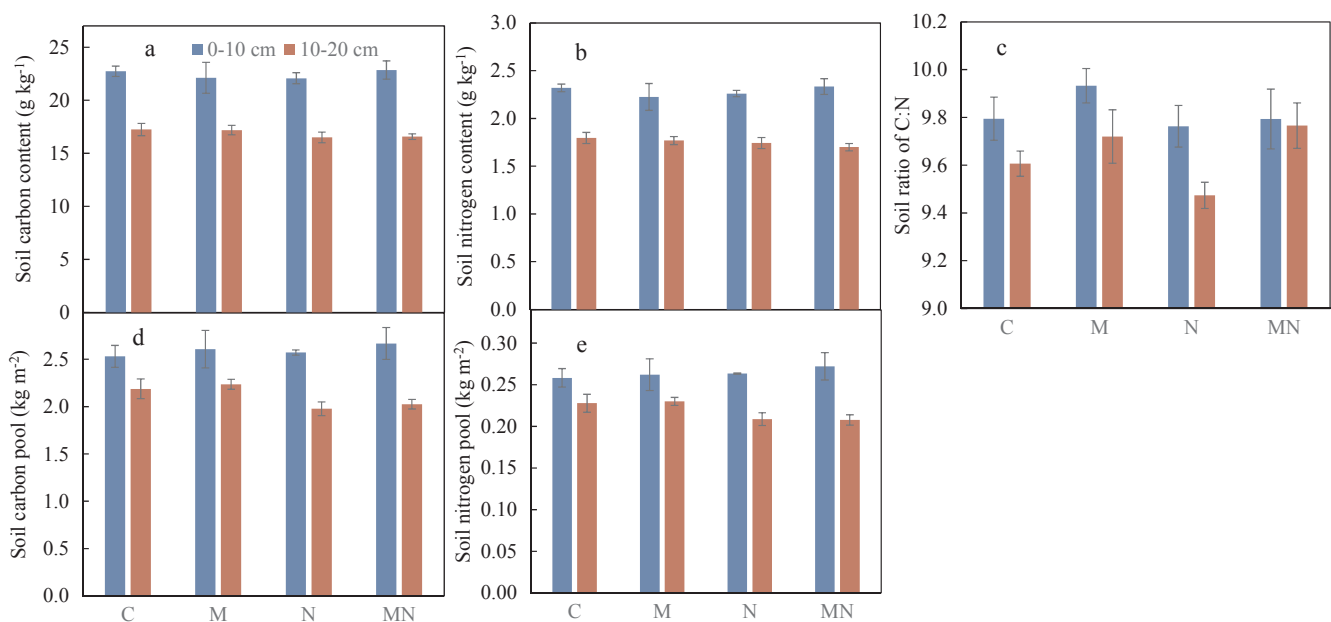


Fig. 4. Soil carbon content (a), soil nitrogen content (b), ratio of soil C:N (c), soil carbon pool (d), and soil nitrogen pool (e) at the soil depth of 0–10 cm and 10–20 cm under the control (C), mowing (M), nitrogen addition (N), and mowing plus nitrogen addition (MN) treatments, respectively. Error bars are the standard errors of the means.

Table 2
Results of variance analysis for soil properties.

F-value	MA	MI	SC	MGD	SOC	MA-OC	MI-OC	SC-OC	SCP	TN	MA-TN	MI-TN	SC-TN	SNP	C:N
M	0.095	0.18	0.007	0.01	0.076	1.326	0.275	0.007	0.558	0.15	1.152	0.461	0.166	0.157	4.124
N	1.487	1.917	0	1.233	0.353	0.904	0.668	0.31	0.797	0.381	0.78	0.791	0.113	0.561	0.837
deep	71.986	75.187	44.119	79.437	135.172	21.449	16.964	94.157	29.553	119.715	20.963	21.515	92.355	26.714	6.455
M * N	0.008	0.005	0.884	0.066	0.154	5.581	0.91	0.432	0.003	0.27	4.729	1.086	0.455	0.002	0.064
M * deep	1.277	2.135	0.417	1.482	0.003	2.586	0.006	0.003	0.042	0.02	2.797	0.024	0.046	0.106	0.703
N * deep	0.602	0.509	0.638	0.942	0.909	2.991	1.115	0.399	2.122	0.7	3.142	1.523	0.592	2.584	0.089
M * N * deep	0.196	0.126	0.218	0.221	1.257	0.059	0.293	0.3	0.002	1.282	0.098	0.326	0.695	0.053	1.023

Bold values were significant at $P < 0.05$.

MA proportion of macroaggregates (%), MI proportion of microaggregates (%), SC proportion of < 0.053 mm size class (%), MGD mean geometric diameter (mm), SOC organic carbon content in total soils (g kg^{-1}), MA-OC organic carbon content in macroaggregates (g kg^{-1}), MI-OC organic carbon content in microaggregates (g kg^{-1}), SC-OC organic carbon content in < 0.053 mm size class (g kg^{-1}), SCP soil organic carbon pool, TN total nitrogen content in total soils (g kg^{-1}), MA-TN total nitrogen content in macroaggregates (g kg^{-1}), MI-TN total nitrogen content in microaggregates (g kg^{-1}), SC-TN total nitrogen content in < 0.053 mm size class (g kg^{-1}), SNP soil total nitrogen pool.

plant N pools in response to N deposition indicates an improvement in the quality of the herbage in the temperate steppe. The stimulation of N addition on plant C and N pools indicates that N fertilization at the $10 \text{ g N m}^{-2} \text{ year}^{-1}$ was a suitable grassland management technique for ecosystem C sequestration and animal husbandry in this grassland.

Mowing significantly decreased shoot and litter mass, but it had no effect on root biomass in this steppe after the 4-year treatment. Improved grassland management (e.g. optimized grazing intensity and mowing frequency) has been found to increase ecosystem C sequestration (Koncz et al., 2017). Mowing for hay production is an alternative management practice, mainly because grassland productivity remains a key ecosystem service (Kitchen et al., 2009). Previous studies have shown that mowing increases plant productivity by creating a more favorable energy environment in a tallgrass prairie (Kitchen et al., 2009). However, our study is similar to results that mowing decreases shoot biomass and litter mass in the temperate steppe (Liu et al., 2018b). The lack of significant mowing effects on root mass (Fig. 1) may be caused by the shift in plant species composition, whereby plants

reallocate resources to replace lost aboveground biomass in a mesotrophic grassland (Heyburn et al., 2017). Moreover, mowing has no effect on total root biomass, it can increase root biomass in the upper 10 cm of soil layer but has little effect at lower depths (Kitchen et al., 2009). Leaf functional traits play a key role in the ecosystem C cycle, and persistent mowing increases plant N content by stimulating the growth of the low stature species through the chronic removal of litter in a temperate steppe (Han et al., 2014). However, the lack of response in terms of plant N content to mowing in this study, which indicates that vegetation removal by mowing once a year has no effect on plant nutrient status, can be illustrated by the non response of soil total N content in this short-term experiment in this steppe. The plant C and N pools showed no response to mowing indicating that annual mowing had no effect on the nutrient biogeochemical cycles in this grassland over the short-term.

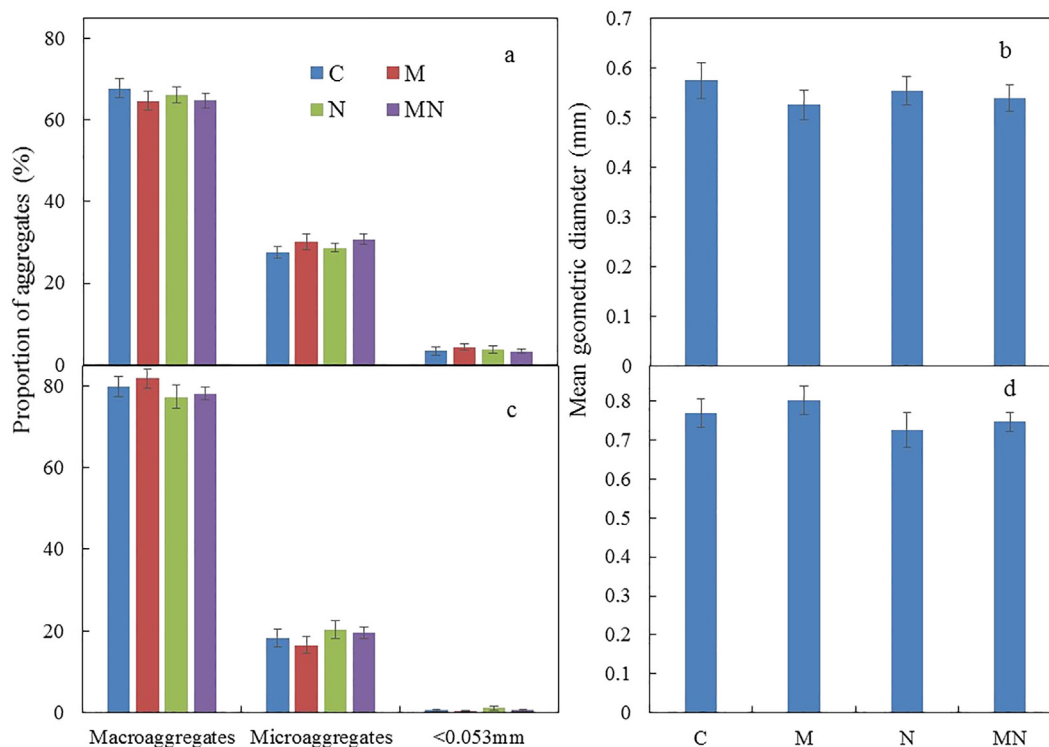


Fig. 5. Proportion of macroaggregates, microaggregates, < 0.053 mm, and mean geometric diameter (mm) at the 0–10 (a, b) and 10–20 (c, d) cm depths under the control (C), mowing (M), nitrogen addition (N), and mowing plus nitrogen addition (MN) treatments. Error bars are the standard errors of the means.

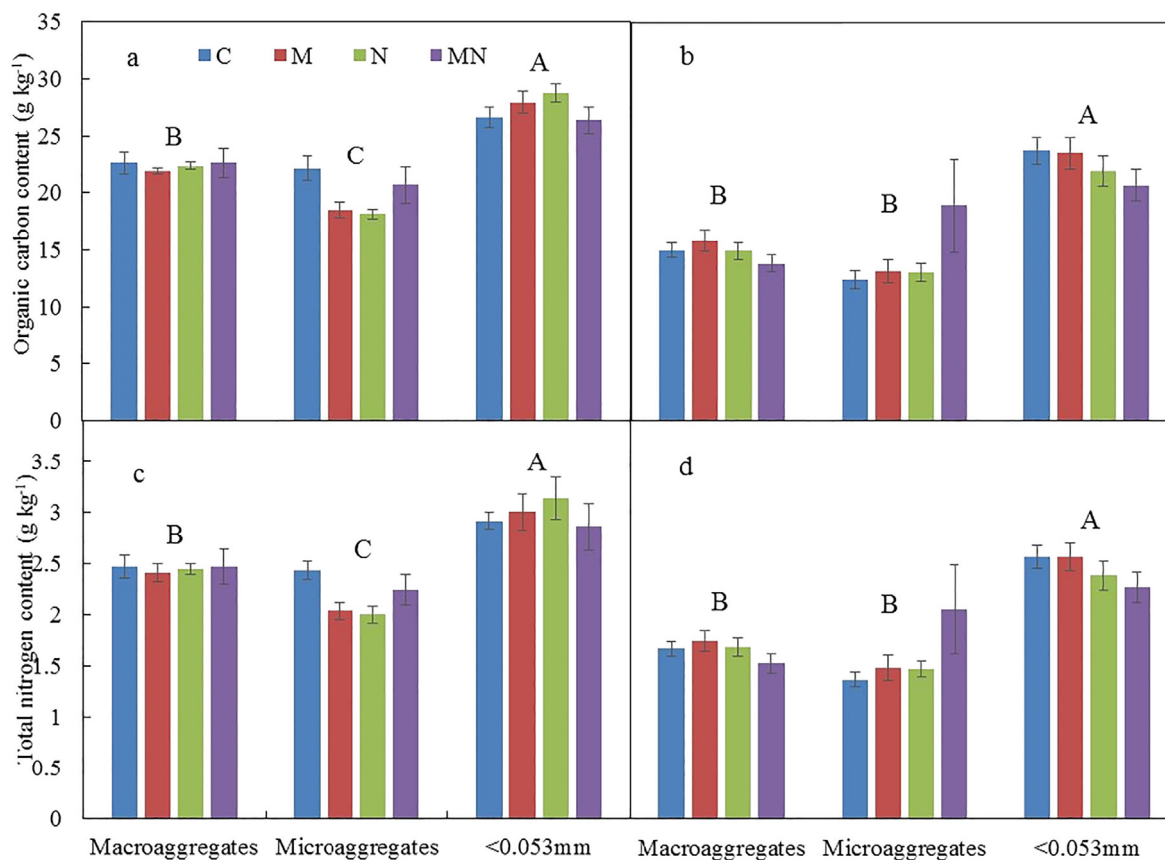


Fig. 6. Aggregate-associated carbon content and nitrogen content at the 0–10 (a, c) and 10–20 (b, d) cm depths under the control (C), mowing (M), nitrogen addition (N), and mowing plus nitrogen addition (MN) treatments. Error bars are the standard errors of the means. Different upper letters indicate there were significant differences among the macroaggregates, microaggregates, < 0.053 mm proportions.

4.2. The responses of soil carbon and nitrogen to treatment

Soil C and N contents and pools showed no response to N addition in this study. A meta-analysis shows that N addition stimulates terrestrial ecosystem C storage (Yue et al., 2016). Moreover, an experiment in a western North American grassland showed that nutrient addition increased soil C content through the stimulation of root and shoot biomass and had no impact on decomposition (Ziter and MacDougall, 2013). However, the finding that adding N failed to alter the soil C and N pools throughout the soil profile in this temperate steppe are similar to the studies conducted in the Loess Plateau (Han et al., 2013) and in Pennsylvania (Skinner, 2013). There were three mechanisms for the lack of response of the soil C and N pools to N addition in this study. First, increasing soil C emissions offset the stimulation on the plant C pool under the N addition treatment. On the one hand, N addition increased the shoot biomass and surface litter mass (Fig. 1) and stimulated the soil C source. However, N addition suppressed the ratio of plant C to N (Fig. 2), and consequently stimulated C decomposition and ecosystem respiration (Niu et al., 2010). Second, N addition changed the dominant species from forb to grass, as the plant functional group affected the ecosystem nutrient cycling (Wang et al., 2014). Third, plant roots are the main source of soil organic matter, but N addition had no effect on root biomass (Kong et al., 2019). Therefore, N addition had no effect on the soil C and N content or pools in this study. Long-term application of fertilizer significantly increased the macroaggregate fraction, mean geometric diameter, and aggregate-associated C and N in a 27-yr experiment in a dry grassland on the Loess Plateau of China (Zhang et al., 2015). However, the soil aggregate proportion, mean geometric diameter and aggregate-associated C and N showed no response to N addition, possibly because of the short duration of the

experiment (Chen et al., 2017). Previous studies have shown that soil structure and organic matter are interrelated, and that the gain or loss of organic matter can induce soil structural changes (Caplan et al., 2017). In this paper, N addition is considered to have no effect on the soil aggregate proportions, given the lack of response of the soil C and N content to N addition treatment.

This study found that mowing has no effect on soil C and N content or pools. The results that positive, negative and neutral responses of soil C content to mowing coexisted. Previous studies have shown that defoliation can increase soil C content and thus increase the plant litter ratio of C to N. Defoliation can also increase resistance to decomposition, stimulate species diversity, and increase photosynthesis and root exudates in grasslands (Cong et al., 2015; Liu et al., 2015; Li et al., 2017). In a tallgrass prairie, mowing was found to decrease the surface soil C and N contents (Kitchen et al., 2009). Moreover, mowing has been shown to have no effect on the soil C content or on the soil microbial biomass in the long term (Kotas et al., 2017). The lack of response of soil C and N content to mowing in our experiment can be explained by three mechanisms. First, the suppression of the soil C and N input caused by the decrease in shoot biomass and litter mass (Fig. 1) decelerated the mineralization of the soil organic matter under the mowing treatment (Shahzad et al., 2012). Second, given that root biomass was about three times as much as that of shoot biomass in the semi-arid grassland (Wang et al., 2014), the lack of response of root C input to the mowing treatment did not affect the soil C content. Third, testing the response of soil C and N content to change in land use requires a long-term experiment (Chen et al., 2017), but this present experiment lasted only four years. Aggregates provide physical protection from microbial decomposition and the loss of C and N (Six et al., 2000; Chen et al., 2019). The results in this study differ from the study

conducted in the Loess Plateau, which found that land use significantly affected the proportions of macroaggregates, soil C content and ratios of C to N (Ge et al., 2019). The lack of response to the mowing treatment in this study on the aggregate proportions, and on the associated C and N, may have been caused by the same root biomass existing in the control plots and the mowed plots. Annual hay harvesting can provide food for large mammals, and it had no effect on ecosystem C and N sequestration in the study region. Given the pivotal role played by grasslands in the global terrestrial carbon cycle, mowing once a year is found to be a suitable grassland management technique in this temperate steppe.

5. Conclusion

In this study, we examined the effects of mowing and N addition on the distribution of C and N in shoots, litter, roots, bulk soil and water-stable aggregates in a temperate steppe. The results showed that N addition significantly increased the N content and N pool, and it decreased the ratio of C to N in shoots, litter and roots. Moreover, N addition stimulated the mass and plant C pool in shoots and litter, respectively. However, neither mowing nor N addition affected the C and N content in bulk soil or aggregates, nor did they affect the mean geometric diameter after a 4-yr experiment in the study region. These results suggest that testing the mechanisms of soil C sequestration by N addition requires a long-term experiment. Plant C and N pools are more sensitive to N addition than they are to mowing, and N deposition may stimulate grassland productivity in the temperate steppe.

Declaration of Competing Interest

The authors declared that there is no conflict of interest.

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Authors' contributions

Y.L. and D.W. designed the research. D.W. collected data. D.W. and Y.L. performed the analysis. All authors wrote the article. All authors contributed critically to the drafts and gave final approval for publication. The authors declare that they have no competing interests.

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