



Effects of long-term mowing on the fractions and chemical composition of soil organic matter in a semiarid grassland

Jiangye Li¹, Qichun Zhang¹, Yong Li¹, Yimeng Liu², Jianming Xu¹, and Hongjie Di¹

¹Zhejiang Provincial Key Laboratory of Agricultural Resources and Environment, Key Laboratory of Environment Remediation and Ecological Health, Ministry of Education, Zhejiang University, Hangzhou 310058, China

²School of Economics and Resource Management, Beijing Normal University, Beijing 100875, China

Correspondence to: Qichun Zhang (qc Zhang@zju.edu.cn)

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Abstract. The grassland ecosystem is a significantly important terrestrial carbon pool. Intensive mowing is common to meet the need of increased livestock. However, little information on the quality and quantity of soil organic matter (SOM) under different mowing managements has been documented. In this work, in order to evaluate the impacts of different mowing managements on the quality and quantity of SOM, the fractions and chemical composition of SOM under different mowing managements were determined using traditional fractionation methods and spectroscopy technologies, including advanced nuclear magnetic resonance (NMR) (e.g. cross-polarization magic angle spinning ¹³C-NMR, CPMAS ¹³C-NMR) and Fourier-transform infrared (FTIR) based on a 13-year field mowing trial with four treatments: unmown (M0), mowing once every second year (M1/2), mowing once a year (M1) and mowing twice a year (M2). The results showed that compared with M0, M1/2 and M1 significantly enhanced the SOM accumulation and increased the stability of SOM by enhancing humification, while M2 limited SOM accumulation and microbial biomass. Substituted alkyl carbon (C) was the major organic C type in the grassland ecosystem, and it made up over 40 % of the total C. M1/2 and M1 significantly increased stable C functional groups (alkyl C and aromatic C) by degrading labile C functional groups (O-alkyl and carbonyl C) and forming recalcitrant humus, while M2 had opposite effects. The consistent increase in the values of NMR indices reflecting the degradation degree, hydrophobicity and aromaticity of SOM in M1 reflected the fact that M1 had the largest contribution to increasing the stability of SOM, while these values in M2 were similar to those in M0. Significant correlations between different SOM frac-

tions and nitrogen (N) mineralization, and between the contents of different C functional groups and net soil organic nitrogen mineralization or microbial biomass C, indicated that the shifts in SOM fractions and chemical composition were closely related to soil microbial biomass and activity. Therefore, in view of the quality and quantity of SOM and the sustainable development of grassland ecosystems, M1 was the optimal mowing management, while M2 should be avoided in the semiarid grassland.

1 Introduction

Soil organic matter (SOM) plays a central role in the global biogeochemical cycles of most major nutrients. The soil C pool is the largest C storage in the terrestrial ecosystem and the organic C pool consists of more than 90 % of the total soil C pool. Grasslands account for 40.5 % of the terrestrial area globally, and it is estimated that 34 % of the global terrestrial organic C was stored in the grasslands (White et al., 2000). In China, grasslands also cover more than 40 % of the terrestrial surface and the Inner Mongolia grassland, which is one of the most important animal husbandry bases, representing more than a quarter of the total grassland area (National Environment Protection Bureau of China, 2006). Therefore, soil C and nitrogen (N) cycling in Inner Mongolia grassland has been a hot topic (Shan et al., 2011; Wang et al., 2014, 2016). Mowing once a year is one of the common practices in grassland ecosystems, and it is reported that mowing once a year increases the stocks of soil C and N by facilitating plant species richness, plant productivity, root biomass and

root exudates (Socher et al., 2012; Cong et al., 2014). However, in order to prepare enough winter feed for the increased livestock, high-frequency mowing is needed, which might result in the reduction of plant species diversity and block soil C and N turnover, as few microbes are able to bear such a degree of disturbance.

Increased plant diversity and enhanced fresh SOC input by mowing once a year can lead to the degradation of recalcitrant organic compounds by the priming effect (Fontaine et al., 2011). In addition, different plant species release diverse organic compounds, and these would have an impact on soil microbial communities (Dijkstra et al., 2005). It was documented that mowing could increase the activity of extracellular enzymes to decompose polymeric C (aromatic polymer from lignin derived from litter or root residue) into monomers (Steinauer et al., 2015), including simple but resistant C like alkyl C, a decomposition product which is stable in soil. The stability of the soil C pool is closely related to the sustainability of soil functions. However, it has been unclear how stable the SOM is under the different mowing managements. Therefore, to better assess the ecological significance of long-term mowing managements, it is necessary to study the impacts of different mowing managements on the quantity and quality of SOM.

Soil organic matter composition is often used to evaluate the stability of soil C pools. The fractions of SOM are traditionally classified based on the assumption of organo-mineral interactions and spatial arrangements of soil particle size by physical methods (Cao et al., 2011) and relying on their solubility in acid or base extractants by chemical methods (Olk and Gregorich, 2006). Generally, labile SOC fractions include water-soluble organic C (WSOC), microbial biomass C (MBC) and readily oxidized C (ROC), which are considered to be early and sensitive indicators of soil quality because they could rapidly respond to soil management practice (Chen et al., 2017), while humus is recalcitrant SOM. These fractions are extracted by different extractants. Spectroscopy is a powerful tool for identifying the chemical structures of SOM as soil samples are measured directly rather than determined after a series of extractions which might alter the nature of SOM. Fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) are widely used to study the chemical composition of organic matter (Olk, 2006; Mao et al., 2008; Zhou et al., 2014). Advanced solid-state NMR, i.e. cross-polarization magic angle spinning ^{13}C -NMR (CPMAS ^{13}C -NMR) applied in characterizing chemical structures of SOM, is also an important approach to reveal the essential changes in SOM formation and degradation in paddy fields and forest ecosystems (Zhou et al., 2014; Panettieri et al., 2014; Zhang et al., 2015) and in litter and wood decomposition processes in these ecosystems (Sanaullah et al., 2012; Bonanomi et al., 2013; Hu et al., 2017). This approach can provide the information of SOM structure noninvasively without using solvents. Generally, alkyl C (45–0 ppm), *N*-akyl/methoxyl C

(60–45 ppm), *O*-alkyl C (90–60 ppm), di-*O*-alkyl C (110–90 ppm), aryl C (140–110 ppm), phenolic C (165–140 ppm) and carboxyl and carbonyl C (210–165 ppm) are identified in detail, from the spectra of ^{13}C -NMR of soil samples (Baumann et al., 2009, 2013; Zhao et al., 2012). This functional C can also be generally grouped into four groups: carbonyl C (210–165 ppm), aromatics C (165–110 ppm), substituted alkyl C (110–45 ppm), and alkyl C (45–0 ppm) (Plaza et al., 2013; Zhao et al., 2012; Boeni et al., 2014). In addition, alkyl, *N*-akyl/methoxyl, aryl and phenolic C are often derived from lignin, while *O*-alkyl and di-*O*-alkyl C are generally included in polysaccharide (carbohydrates) (Preston et al., 1998; Bonanomi et al., 2013). By analysing the functional C composition, the nature of SOM can be better understood and the quality and quantity of SOM can be more exactly evaluated. However, the cost of solid-state ^{13}C -NMR is high, especially for complex soil samples, because of the length of time it takes to identify the chemical structure.

To better understand and evaluate the quality of SOM, elemental analysis (EA) and FTIR are often combined to help get more accurate information (Mao et al., 2008; Zhou et al., 2014). In grassland ecosystems, these tools are also used to study SOM stocks and quality (Baumann et al., 2016; Knicker et al., 2012). However, there are no reports of the effects of mowing and mowing frequency on the chemical structure of the whole SOM, which reflects the nature of SOM. In this study, we combined advanced solid-state NMR with traditional methods to investigate the quality and quantity of the grassland soil organic C under different mowing managements. The objective of this study was to investigate the impacts of long-term mowing practices on the chemical composition of SOM and evaluate the stability of the grassland soil carbon pools under different mowing frequencies.

2 Materials and methods

2.1 Site description and experimental design

The study site was located in the Xilingol region of Inner Mongolia (43°269′–44°089′ N and 116°049′–117°059′ E) in northern China. It has a temperate semiarid climate, with an annual mean temperature of 0.5 °C and annual average precipitation of 350 mm, most of which falls during the summer. The annual potential evapotranspiration ranged from 1600 to 1800 mm. The soil was Calcic-orthic Aridisol according to the US soil taxonomy (or sandy-loam dark chestnut soil in the Chinese classification system) (Baoyin et al., 2014) and, in the profile, there was a humus layer of 20–30 cm and a calcic layer at ca. 50 cm depth (Jiang, 1988). The characteristic vegetation of this region was *Leymus chinensis* (*L. chinensis*), accounting for 55 ± 15 % (mean ± standard deviation) of total herbage yield. Other species in order of decreasing proportion of total herbage yield are tall bunchgrasses (mostly *Stipa grandis* and *Agropyron michnoi*), short

bunchgrasses (*Cleistogenes squarrosa* and *Koeleria cristata* (L.) Schrad) and sedge (*Carex korshingski* Kom.), forbs and legumes (Baoyin et al., 2014). The growing season usually started in May and ended in September.

The long-term mowing experiment has been carried out since 2001 in a permanent enclosure by the Inner Mongolia Grassland Ecosystem Research Station of the Chinese Academy of Sciences. The enclosure for the mowing experiment was divided into 12 plots (24 m × 20 m for each plot). There were four treatments, each with three replicates. The four treatments were unmown (M0), mowing once every two years (M1/2) in August, mowing once a year (M1) in August when aboveground biomass of *L. chinensis* reached the peak, and mowing twice every year (M2) in June when the palatability of *L. chinensis* was best for the livestock and in September when *L. chinensis* was withered.

2.2 Soil sample collection

Soil samples were collected from 0 to 10 cm depth using a soil auger (7 cm in diameter and 10 cm in depth) in October 2013 at the end of the growing season and all plots experienced the grass cutting in this year. Five soil cores were collected from each plot at random locations and they were combined and mixed thoroughly to form a composite sample. Visible roots and litter residues and large soil fauna in the soil samples were removed. The soil samples of around 1 kg were put into ziplock bags and transported to the lab on ice quickly. In the lab, the soil samples were passed through a 2 mm sieve and subsampled into two parts. One part was air-dried for basic physical and chemical properties analysis; the other fresh part was used to analyse the fractions and chemical composition of SOM as soon as possible and, if this part could not be analysed in a short time, they were stored at -20°C to avoid the impacts of storage temperature on the indicators determined, especially the microbial indicators.

2.3 Measurements of bulk soil basic properties

Soil pH was measured using a water-to-soil ratio of 2.5 : 1. Soil moisture content was determined by oven drying for 16 h to a constant mass at 105°C . The content of soil organic C (SOC) and total nitrogen (TN), alkali-hydrolysable N (AN), Olsen phosphorus (Olsen P) and net N mineralization was determined, referring to Kalembasa and Jenkinson (1973), Bremner (1965), Bao (2000) and Lin (2010), respectively.

2.4 Soil organic matter fractionation

Soil microbial biomass carbon (MBC) was extracted using the chloroform fumigation extraction (Vance et al., 1987; Wu et al., 1990) and determined using a TOC analyser (Elementar Liqui TOC, Elementar Co., Hanau, Germany). Water soluble organic carbon (WSOC) was determined using a modified method (Li et al., 2013). Briefly, WSOC was extracted from 5.0 g of fresh soil using a soil-to-water ratio of 1 : 10

at 25°C , and shaken for 30 min at a speed of 250 rpm. The samples were subsequently centrifuged ($1146 \times g$, 20 min), and then the supernatant was filtered using a $0.45 \mu\text{m}$ membrane filter. The filtrate was measured by the same TOC analyser mentioned above. Soil readily oxidizable carbon (ROC) was determined and calculated following the detailed procedure described by Li et al. (2013). The mobile humic acid (MHA) and calcium humic acid (CaHA) were extracted following the procedure by Mao et al. (2008). Thirty grams of air-dry soil was used to extract the two humic fractions and the extracted humic fractions were freeze-dried using a freeze-drying machine (FD-1C-50, Beijing, China), and then weighted, respectively.

2.5 Analysis of the chemical composition of soil organic matter (SOM)

To remove paramagnetic materials (Fe^{3+} , Mn^{2+}) and increase the signal-to-noise ratio, the soil samples were pre-treated with HF (10 %, v/v) using the procedure detailed in Li et al. (2010), and finally, the SOM samples were freeze-dried. It is reported that the chemical composition of SOM was not altered as the C / N was similar before and after the HF processing (Mao et al., 2008; Zhou et al., 2014), as was the case in this study (Table S2 in the Supplement), and the C and N content in 10 % HF-treated SOM samples and bulk soil samples was measured using a CHNS Elemental Analyzer (Carlo Erba model EA1108, Italy Vario).

2.5.1 Elemental analysis

The elemental composition of 10 % HF-treated SOM samples was determined using the same CHNS Elemental Analyzer mentioned above. The content of O was estimated as the ash-free mass less C, H, and N. Ash content was determined by combustion overnight in a muffle furnace at 500°C (Ussiri and Johnson, 2003).

2.5.2 FTIR analysis

The FTIR analysis of the SOM samples was conducted on an Avatar 370 FTIR spectrometer (Thermo Nicolet, USA). Each sample was prepared by grinding a 2 mg freezing-drying SOM sample with 200 mg oven-dried KBr in a vibrating puck mill, and then mixtures of about 150 mg were compressed into a translucent pellet using a hydraulic compressor. The pellet was immediately placed on the sample holder, and all spectra ranging from 4000 to 400 cm^{-1} were recorded under the conditions of 4 cm^{-1} wave number resolution, 25 scans, and pure KBr spectra as background (Zhou et al., 2014). Absorption peaks or bands were assigned to organic functional groups following Zhou et al. (2014). Only peaks or bands in the functional group region from 4000 to 1000 cm^{-1} of FTIR spectra were assigned because peaks in the fingerprint region below 1000 cm^{-1} were difficult to as-

Table 1. Calculation formulas of different ^{13}C -NMR indices.

Index	Formula	Reference
A / OA	alkyl C (45–0 ppm) / <i>O</i> -alkyl C (110–60 ppm)	Mathers et al. (2007)
CC / MC	carbohydrate C (90–60 ppm) / methoxyl C (60–45 ppm)	Zhao et al. (2012)
HB / HI	hydrophobic C (45–0 ppm + 165–110 ppm) / hydrophilic C (110–60 ppm + 210–165 ppm)	Spaccini et al. (2002)
Aliphaticity, %	(alkyl C + Substituted C) · 100 / (alkyl C + substituted C + aromatic C)	Zhao et al. (2012)
Aromaticity, %	aromatic C · 100 / (alkyl C + substituted C + aromatic C)	
Al / Ar	aliphaticity / aromaticity	
Lignin C	phenolic C · 4.5 + methoxyl C	Preston et al. (1998)
Polysaccharide C	1.2 · (<i>O</i> -alkyl C – phenolic C · 1.5)	
L / P	lignin C / polysaccharide C	

sign and were very complex, usually overlapping with signals of inorganic soil minerals.

2.5.3 Solid-state CPMAS ^{13}C -NMR analysis

A solid-state ^{13}C -NMR experiment was performed on a Bruker Avance II 300 (Bruker Instrumental Inc) equipped with a 7 mm CPMAS (cross-polarization magic-angle-spinning) detector. NMR spectra were acquired under the conditions of a spectrometer frequency of 75 MHz, a MAS spinning frequency of 5000 Hz, a recycle time of 2.5 s and a contact time of 2 ms. The external standard used for chemical shift determination was hexam-ethylbenzene (methyl at 17.33 ppm). The quantified contribution of each type of C to the total signal intensity and promotion in CPMAS ^{13}C -NMR spectrum was automatically integrated after the separation of hexam-ethylbenzene to calculate the area of the peaks which appeared in the corresponding chemical region using MestreNova software 8.1.0 (Mestrelab, Research Inc). The ^{13}C -NMR spectrum was assigned into seven regions as the previous studies (Baumann et al., 2009, 2013; Zhao et al., 2012) and they were grouped into four main chemical environments according to the ^{13}C nucleus: carbonyl C (210–165 ppm), aromatics C (165–110 ppm), substituted alkyl C (110–45 ppm), alkyl C (45–0 ppm) (Plaza et al., 2013; Zhao et al., 2012; Boeni et al., 2014). The seven assignments of ^{13}C -NMR spectrum and potential sources of functional groups in each assignment were showed in Table S1. To better evaluate the quality of C pools, some indices were calculated following the formula in Table 1.

2.6 Data analysis

Data were statistically analysed using SPSS 21.0 by one-way analysis of variance (ANOVA), and means were separated by Duncan's multiple range test at 5 % level. The figures were created using Origin 8.1 and the data were the mean values ($n = 3$). Linear regression analysis was conducted after the Pearson product–moment correlation analysis by a two-tailed test in SPSS 21.0 using the data in all mowing treatments ($n = 9$) except for the unmown (M0).

3 Results

3.1 Basic properties of bulk soil and net N mineralization

Soil pH was around 7.3 and was not affected by long-term mowing (Table 2). However, long-term mowing had a significant impact on soil nutrient concentrations. Compared with M0 (unmown), mowing once every second year (M1/2) and mowing once a year (M1) significantly increased SOC content ($P < 0.05$) while the SOC content in M2 was similar to that in M0 ($P > 0.05$). The TN content in M1 was the highest and significantly higher than that in treatment M2. The total N content in M2 was also significantly lower than those in the other two treatments (M1/2 and M0). Soil Olsen P contents in all the treatments were very low, around 1.2 mg kg^{-1} , and no significant difference was observed between the treatments ($P > 0.05$). The AN content in the soil in M2 was significantly lower than those in the other treatments ($P < 0.05$), while there was no significant difference between the other treatments ($P > 0.05$). Net N mineralization in M1 was significantly greater than that in the other treatments, and it was significantly lower in M2 than that in other treatments ($P < 0.05$).

3.2 Soil organic matter fractions

Long-term mowing had major impacts on labile C and recalcitrant SOM (Table 3). Compared with M0, M1/2 significantly increased soil MBC content, while M2 significantly decreased soil MBC content ($P < 0.05$). WSOC and ROC contents in all mowing treatments were significantly 50 % lower than those in M0 ($P < 0.05$). Among different mowing treatments, no difference was observed in soil WSOC content ($P < 0.05$), while the soil ROC content in M2 treatment was significantly lower than that in M1 ($P < 0.05$). The total content of both humic fractions (MHA and CaHA) accounted for a major proportion of SOM, especially in M1, where it reached 73.0 % (Table 3), and this was significantly higher than that (53.1 %) in M2 ($P < 0.05$). The CaHA content was about 2–4 times that of MHA across all treatments.

Table 2. Basic description of soil properties under different mowing treatments.

Treatment	pH	SOC	TN	Olsen P	AN	Net N mineralization
	H ₂ O	g kg ⁻¹		mg kg ⁻¹		mg N g ⁻¹
M0	7.3 ± 0.1 a	17.9 ± 0.6 b	1.5 ± 0.0 ab	1.0 ± 0.15 a	75 ± 0.59 a	194 ± 3.76 b
M1/2	7.3 ± 0.0 a	20.2 ± 1.6 a	1.5 ± 0.4 ab	1.3 ± 0.06 a	86 ± 1.42 a	176 ± 7.51 b
M1	7.3 ± 0.1 a	21.7 ± 0.3 a	1.7 ± 0.0 a	1.2 ± 0.03 a	86 ± 0.00 a	225 ± 2.51 a
M2	7.2 ± 0.0 a	17.8 ± 0.8 b	1.3 ± 0.0 b	1.2 ± 0.03 a	57 ± 0.00 b	127 ± 7.50 c

M0, unmown; M1/2, mowing once every second year; M1, mowing once a year; M2, mowing twice a year. The value was the mean ± S.E., *n* = 3. SOC, soil organic carbon; TN, total nitrogen; Olsen P, Olsen phosphorus; AN, alkali-hydrolysable nitrogen; net N mineralization, net nitrogen mineralization. Different lowercase letters in the same column indicated that the difference between treatments reaches a 5 % significance level.

Table 3. Effect of different mowing managements on bulk SOM fractions.

Treatment	MBC	WSOC	ROC	MHA	CaHA	HA / SOM
	mg kg ⁻¹			g kg ⁻¹		%
M0	139.0 ± 9.81 b	98.6 ± 9.42 a	7.3 ± 0.65 a	6.0 ± 0.76 a	14.0 ± 0.87 b	64.8 a
M1/2	167.9 ± 3.70 a	42.4 ± 3.51 b	3.1 ± 0.17 bc	4.6 ± 0.76 a	20.5 ± 0.53 a	72.2 a
M1	144.6 ± 8.09 b	45.6 ± 2.37 b	3.5 ± 0.20 b	6.0 ± 0.55 a	21.5 ± 0.46 a	73.0 a
M2	101.3 ± 6.23 c	38.8 ± 5.51 b	2.3 ± 0.12 c	3.9 ± 0.57 b	12.9 ± 0.89 b	53.1 b

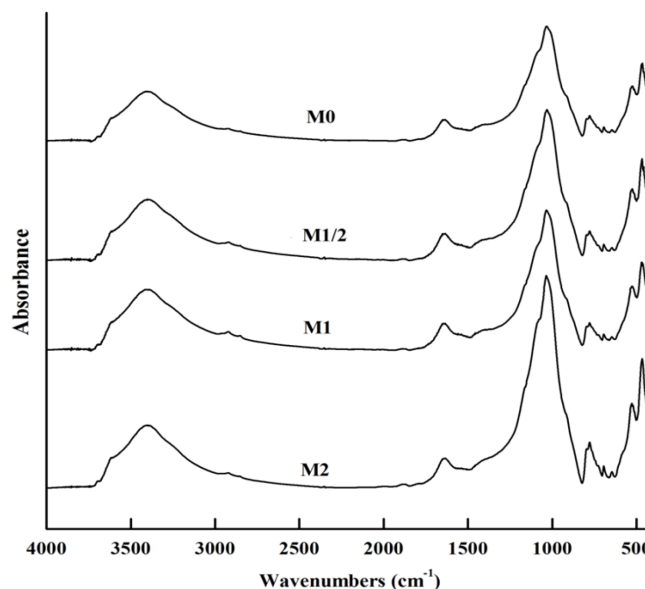
M0, unmown; M1/2, mowing once every second year; M1, mowing once a year; M2, mowing twice a year. The value was the mean ± S.E., *n* = 3. WSOC, water soluble organic carbon. MBC, microbial biomass carbon. ROC, readily oxidized carbon. MHA, mobile humic acid. CaHA, calcium humic acid. SOM, soil total organic matter. HA = MHA + CaHA.

Compared with M0, M2 significantly decreased MHA content ($P < 0.05$) but did not affect CaHA content significantly ($P > 0.05$). However, M1 and M1/2 significantly increased CaHA content ($P < 0.05$) but did not significantly affect MHA content. Thus, both MHA and CaHA contents in soils of M1/2 and M1 were significantly higher than that in M2 ($P < 0.05$).

3.3 Chemical structure of SOM

Parameters of the elemental composition of the SOM were shown in Table 4. The content of hydrogen (H) and oxygen (O) varied from 0.49 to 0.63 % and from 0.25 to 0.35 %, respectively. Compared with M0, all mowing treatments significantly decreased the H content, and M2 also significantly decreased O content ($P < 0.05$). The ratios of H / C and O / C varied from 0.13 to 0.16 % and from 0.06 to 0.09 %, respectively, and the H / C and O / C ratios in M1/2 and M1 were significantly lower than M2 or M0 ($P < 0.05$).

The FTIR spectra of the SOM extracted from the grassland soil under different mowing treatments were shown in Fig. 1. The spectra were dominated by the broad peak around 3406 cm⁻¹, sharp peaks around 1030 cm⁻¹ and medium sharp peaks around 1653 cm⁻¹, which were ascribed to O–H stretching in alcohols, carboxylic acids and phenols, C–OH stretching in carbohydrates, and C=C stretching in aromatics, respectively. The intensity of other peaks in the FTIR spectra was relatively low. Small peaks at 2928 and closing to 1500 cm⁻¹ due to aliphatic C–H stretching in CH₂ / CH₃

**Figure 1.** FTIR spectra of bulk SOM under different long-term mowing managements.

and amide N–C / amino–NH vibrations, and aliphatic C–H bending in CH₂ / CH₃, respectively, were found in all treatments. However, only some small differences in the intensity of the peaks shown in the FTIR spectra were shown qualitatively between different treatments. The intensity of the peak at 2928 cm⁻¹ in M1 was stronger than that in M0 and

Table 4. Elemental composition of SOM from surface soils in grassland soil with different mowing frequencies.

Treatment	Elemental composition, %				Atom ratios	
	C	H	N	O	H/C	O/C
M0	3.94 ± 0.03 b	0.63 ± 0.01 a	0.38 ± 0.01 b	0.35 ± 0.02 a	0.16 a	0.09 a
M1/2	3.95 ± 0.04 b	0.51 ± 0.02 b	0.39 ± 0.01 b	0.28 ± 0.02 ab	0.13 b	0.07 b
M1	4.32 ± 0.05 a	0.56 ± 0.02 b	0.41 ± 0.02 a	0.26 ± 0.01 ab	0.13 b	0.06 b
M2	3.28 ± 0.03 c	0.49 ± 0.01 b	0.27 ± 0.02 c	0.25 ± 0.02 b	0.15 a	0.08 a

M0, unmown; M1/2, mowing once every second year; M1, mowing once a year; M2, mowing twice a year. The value was the mean ± S.E., $n = 3$.

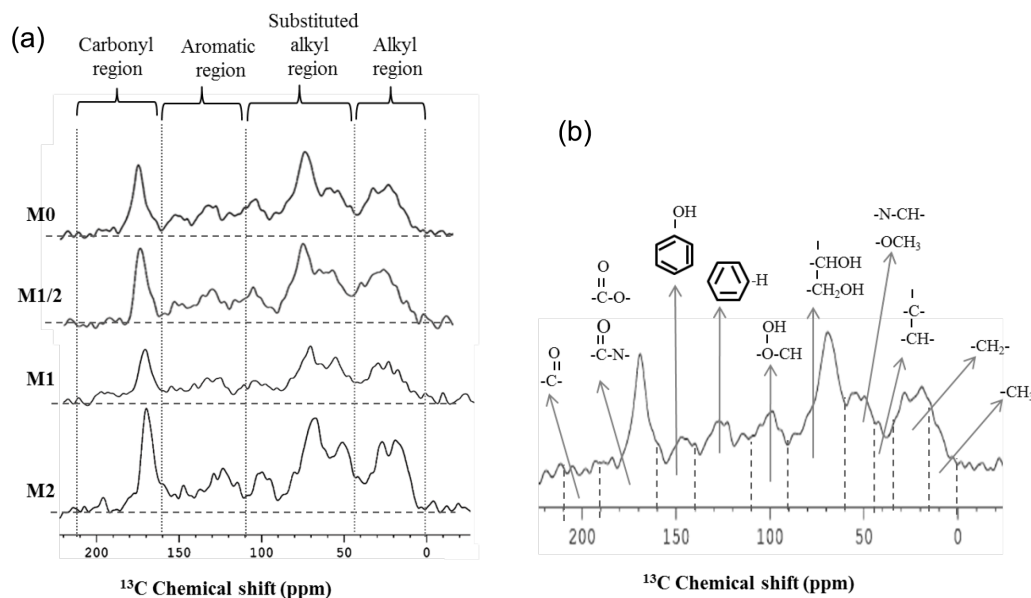


Figure 2. CPMAS ^{13}C -NMR spectra of 10 % HF pretreated SOM. **(a)** CPMAS ^{13}C -NMR spectra of 10 % HF pretreated SOM under different long-term mowing managements. **(b)** Detailed C functional groups in different chemical shifts.

M2, while the intensity of the peak at 1030 cm^{-1} in M1 was weaker than that in M0 and M2 treatments (Fig. 1 and Table 3).

Figure 2 showed the ^{13}C -NMR spectra of the SOM extracted from the grassland soil with different mowing managements (Fig. 2a) and the detailed C functional groups represented by the peaks in the ^{13}C -NMR spectra (Fig. 2b) were shown. In all spectra, the alkyl C (45–0 ppm) and substituted alkyl C (110–45 ppm) peaks were dominant components in SOC composition across all the treatments, accounting for 24.6–27.9 and 41.5–47.6 % of the total spectral fractions, respectively (Table 5 and Fig. 2), followed by aromatic C (165–110 ppm) and carbonyl C (210–165 ppm) peaks, accounting for 16.3–19.1 and 9.3–13.7 % of the total spectral fractions, respectively. In the substituted alkyl C, *O*-alkyl C (90–60 ppm) was the main fraction, making up more than 50 % of the substituted alkyl C, while di-*O*-alkyl (110–90 ppm) only accounted for less than 21 % of the substituted alkyl C, and *N*-alkyl/methoxy C was medium. Compared to M0, mowing significantly increased alkyl C but significantly

decreased substituted alkyl C (except for *N*-alkyl/methoxy C, $P < 0.05$) mainly existing in carbohydrates (Table S1). The proportion of aromatic C (aryl and *O*-aryl C, 165–110 ppm) in M1/2 and M1 was significantly higher than that in M0, while the proportion of carbonyl C (210–165 ppm) in these two treatments was significantly lower than that in M0 ($P < 0.05$). Among mowing treatments, alkyl C, substituted C and aryl C in M2 were significantly lower than those in M1/2 and M1, while *O*-aryl C and carbonyl C in M2 were significantly higher than those in M1/2 and M1 ($P < 0.05$). The *O*-alkyl C in M2 was the lowest among all treatments, which was also consistent with the results of FTIR.

Soil ^{13}C -NMR indices reflecting soil quality directly or indirectly were calculated and the results were shown in Table 6. Soil lignin C, the A / OA ratio and the HB / HI ratio in M0 were significantly lower than those in the mowing treatments ($P < 0.05$), while the CC / MC ratio in M0 was significantly higher than that in mowing treatments ($P < 0.05$). Aliphaticity in M1/2 and M1 was significantly lower than that in M0 and M2, while aromaticity was just the opposite

Table 5. Percentages of total special spectral areas of different functional groups obtained by quantitative CPMAS ^{13}C -NMR for soil samples from grassland soil with different mowing frequencies (%).

Treatment	Alkyl C	Substituted alkyl C				Aromatics		Carbonyls
	45–0 ppm Alkyl	60–45 ppm <i>N</i> -alkyl/methoxyl	90–60 ppm <i>O</i> -alkyl	110–90 ppm di- <i>O</i> -alkyl	140–110 ppm Aryl	165–140 ppm <i>O</i> -aryl	210–165 ppm Carboxyl and carbonyl	
M0	24.6 ± 0.12 c	11.2 ± 0.06 c	27.0 ± 0.17 a	9.4 ± 0.06 a	11.7 ± 0.08 b	4.6 ± 0.04 c	11.1 ± 0.17 b	
M1/2	27.6 ± 0.20 a	12.9 ± 0.05 b	22.5 ± 0.06 b	8.6 ± 0.09 b	13.4 ± 0.09 a	5.7 ± 0.06 b	9.3 ± 0.06 c	
M1	27.9 ± 0.23 a	13.4 ± 0.06 a	22.3 ± 0.15 b	8.1 ± 0.06 c	13.6 ± 0.06 a	5.6 ± 0.06 b	9.1 ± 0.06 c	
M2	25.4 ± 0.12 b	12.3 ± 0.25 bc	21.7 ± 0.09 c	7.5 ± 0.07 c	11.8 ± 0.12 b	6.6 ± 0.07 a	14.7 ± 0.15 a	

M0, unmown; M1/2, mowing once every second year; M1, mowing once a year; M2, mowing twice a year. The value was the mean ± S.E., $n = 3$.

Table 6. CPMAS ^{13}C -NMR indices of SOM from surface soils in grassland soils with different mowing frequencies.

Treatment	Lignin-C	Polysaccharide-C	L / P	Aliphaticity	Aromaticity	Al / Ar	A / OA	HB / HI	CC / MC
	% of SOC			%					
M0	36.4 c	13.9 b	2.61 b	79.3 a	20.7 c	3.84 a	0.91 b	0.76 b	2.23 a
M1/2	38.6 b	16.7 a	2.30 c	78.9 b	21.1 b	3.74 b	1.23 a	0.88 a	1.74 b
M1	38.2 b	16.9 a	2.26 c	78.5 c	21.5 a	3.65 c	1.25 a	0.87 a	1.66 c
M2	42.0 a	14.2 b	2.97 a	79.4 a	20.6 c	3.85 a	1.17 b	0.81 ab	1.76 b

M0, unmown; M1/2, mowing once every second year; M1, mowing once a year; M2, mowing twice a year. L / P, lignin / polysaccharide. A / OA, alkyl C / *O*-alkyl C. HB / HI, hydrophobic C / hydrophilic C. CC / MC, carbohydrate C / methoxyl C. Al / Ar, aliphaticity / aromaticity.

($P < 0.05$), which resulted in an Al / Ar ratio in M1/2 and M1 that was significantly lower than that in M0. There was no difference in aliphaticity, aromaticity and Al / Ar ratios between M0 and M2 ($P > 0.05$). Among different mowing treatments, most of the ^{13}C -NMR indices in M1/2 are similar to M1, except that aromaticity in M1/2 was significantly lower than that in M1, while aliphaticity and the CC / MC ratio in M1/2 were significantly higher than those in M1 ($P < 0.05$). In all of the indices, lignin C, the L / P ratio and aliphaticity in M2 were significantly higher than those in both M1/2 and M1, and the other indices were the opposite ($P < 0.05$).

3.4 Variations of SOM fraction and the C functional group in relation to SOM mineralization and microbial characterization

Soil organic matter content was significantly and positively correlated with MBC, MHA, CaHA and net N mineralization with $r = 0.45, 0.48, 0.89,$ and 0.54 ($P < 0.05$), but was not correlated with WSOC and ROC ($P > 0.05$) (Table 7). ROC was significantly correlated with WSOC, MBC, MHA, CaHA and net N mineralization ($r = 0.55$ – 0.92 , $P < 0.05$) and MHA was significantly correlated with CaHA ($r = 0.82$, $P < 0.05$). Moreover, positive correlations were found between net N mineralization and MBC, MHA, and CaHA, with $r = 0.60, 0.83,$ and 0.75 , respectively ($P < 0.05$).

The relationships between net N mineralization or MBC and the C functional groups of SOC were shown in Table 8. The results showed that N mineralization was related to the

chemical structure of SOC and to microbial biomass. Net N mineralization was not significantly related to four detailed CPMAS ^{13}C -NMR regions (*N*-alkyl/methoxyl C, *O*-alkyl C, di-*O*-alkyl C and aryl C), with $r = 0.28, 0.37, 0.47$ and -0.24 , respectively ($P > 0.05$), but was negatively correlated with *O*-aryl C ($r = -0.94$, $P < 0.001$) and carbonyl C ($r = -0.79$, $P < 0.01$) and the integrated aromatics including aryl C and *O*-aryl C ($r = -0.81$, $P < 0.01$). Consistent with net N mineralization, significant negative correlations were also found between MBC and *O*-aryl C ($r = -0.84$, $P < 0.001$), carbonyl C ($r = -0.96$, $P < 0.01$) and the integrated aromatics ($r = -0.39$, $P < 0.05$). However, both net N mineralization and MBC were positively correlated with alkyl C, with $r = 0.46$ and 0.59 , respectively ($P < 0.05$). Different from net N mineralization, MBC was also significantly correlated with di-*O*-alkyl C and aryl C, with $r = 0.59$ and 0.73 ($P < 0.05$), respectively, but was not correlated with *N*-alkyl/methoxyl C and *O*-alkyl C.

4 Discussion

4.1 SOM accumulation impacted by different mowing practices for the long term

Our results showed that 12-year M1/2 and M1 significantly enhanced SOM accumulation and increased the soil TN content (Table 2), which agreed with previous studies (Cong et al., 2014). Mowing (M1) enhanced plant species by increasing the subordinate plants (Mariotte et al., 2015; Socher et

Table 7. Linear correlation coefficients for relationships among different SOM fractions and net N mineralization.

	SOC	WSOC	MBC	ROC	MHA	CaHA	Net N mineralization
SOC	1						
WSOC	0.11	1					
MBC	0.45	0.37	1				
ROC	0.36	0.90	0.55	1			
MHA	0.48	0.43	0.45	0.92	1		
CaHA	0.89	0.41	0.34	0.81	0.82	1	
Net N mineralization	0.54	0.08	0.60	0.91	0.83	0.75	1

$n = 9$. The bold denotes the difference was significant at the level of $P < 0.05$. SOC: soil total organic carbon. The others were the same as Table 4.

Table 8. Summary of the linear correlation for relationships between net N mineralization, MBC and all the C functional groups of SOC determined by CPMAS ^{13}C -NMR.

Chemical shifts, region (ppm)	Net N mineralization		MBC	
	r	P	r	P
Detail assignments				
Alkyl C (45–0)	0.46	0.047	0.59	0.039
<i>N</i> -alkyl/methoxyl C (60–45)	0.28	0.615	0.29	0.891
<i>O</i> -alkyl C (90–60)	0.37	0.429	0.27	0.992
di- <i>O</i> -alkyl C (110–90)	0.47	0.326	0.59	0.027
Aryl C (140–110)	–0.24	0.798	0.73	0.011
<i>O</i> -aryl C (165–140)	–0.94	< 0.001	–0.84	< 0.001
Carbonyl C (210–165)	–0.79	0.005	–0.96	0.003
Integrated regions				
Unsubstituted alkyl C (45–0)	–	–	–	–
Substituted alkyl C (110–45)	0.70	0.010	0.68	0.014
Aromatics (165–110)	–0.81	0.008	–0.39	0.042
Carbonyls (210–165)	–	–	–	–

$n = 9$. Substituted alkyl C was integrated into *N*-alkyl/methoxyl C, *O*-alkyl C and di-*O*-alkyl C. Aromatics integrated aryl C and *O*-aryl C. In the integrated regions, unsubstituted alkyl C and carbonyls were the same as alkyl C and carbonyl C in detailed assignments, respectively.

al., 2012). Enhanced plant species richness promoted plant productivity and photosynthesis, and thus increased soil carbon and nitrogen stocks in grasslands by more input of organic C and N derived from more root biomass, root exudates and N retention and photosynthetic products (Cong et al., 2014; Gao et al., 2008), which further had a positive feedback to plant productivity, including legume. Legume was common in grassland and moderate mowing would stimulate its productivity to increase atmospheric N fixation (Cardinale et al., 2012), and N enrichment benefited C accumulation, in turn (Riggs and Hobbie, 2016). In addition, the significant increase in CaHA content in M1/2 and M1 was the main and direct reason for SOM accumulation, as the CaHA was the dominant fraction of SOM (Table 3), which indicated that M1/2 and M1 enhanced the humus formation. Moderate mowing increased the fungal community abundance and diversity (Li et al., 2017), and it was reported that fungi could make the molecular structure of humus more complex (Li, 2012).

Compared to moderate mowing, long-term excessive mowing practice resulted in herbage productivity decline due to high nutrient removal from the soil and plant species reduction (Baoyin et al., 2014), which would result in the decrease in labile SOM fractions (WSOC, MBC and ROC) and relatively labile C (MHA) contents in M2. Microbes were sensitive to perturbation and thus MBC was regarded as a reliable indicator of the change in SOC pools caused by management practices (Fang et al., 2009). The significant reduction of MBC content was the key biotic reason for soil net N mineralization reduction (Table 4). Therefore, long-term M2 treatment hampered the soil nutrient cycling and balance, and should be avoided.

4.2 Stability of SOM impacted by different mowing treatments

Different mowing treatments had diverse impacts on the chemical structure of SOM. The composition of SOM chemical structure directly reflected the stability of SOM and thus informed the degradability of SOM. The elemental analysis suggested that long-term mowing practice had major impacts on the elemental composition of SOM. The lower H/C ratio indicated more aromatic compounds or higher aromaticity and saturability, and the higher O/C ratio indicated more carboxyl groups, phenol or carbohydrates with oxygen (Ma et al., 2001; Steelink, 1985; Kim et al., 1991). ^{13}C NMR apparently differentiated the lignin C (including alkyl, *N*-alkyl and aryl C) and carbohydrate C (including *O*-alkyl, di-*O*-alkyl, carbonyl and carboxyl C) (Hu et al., 2017). Therefore, both elemental analysis and the quantified analysis of ^{13}C NMR spectra showed that M1/2 and M1 led to a significant loss of the carbohydrates and accumulation of lignin by more litter input, which indicated that M1/2 and M1 benefited the stability of SOM. Previous studies also reported that aryl C at 140–110 ppm was rich in condensed aromatics and was quite stable in the soil, and its content could reflect the stability of C pools (Zhou et al., 2014). According to this conclusion, M2 had little influence on the stability of SOM. However, the highest content of carbonyl and carboxyl C in M2 suggested

that SOM in M2 was not stable, as compounds that included carbonyl and carboxyl C could be degraded relatively easily.

The accumulation of lignin and the increase in microbial biomass were the favourable conditions of humification. Therefore, the CaHA fraction in SOM increased by 46.9–52.5 % after 12-year M1/2 and M1. This suggested that long-term moderate mowing managements enhanced the degree of humification of SOM. By contrast, the reduction of litter input and the significantly decreased microbial biomass in M2 led to the reduction of humus. Zech et al. (1997) also documented that excessive human activity resulted in the humic horizons' disappearance in many tropical regions. This suggested that M2 hindered SOM humification and disturbed the SOM balance, which might be because the plant diversity and productivity were limited (Socher et al., 2012; Mariotte et al., 2013), resulting in lower labile carbon content and less soil microbial function (Steinauer et al., 2015).

In the CPMAS ^{13}C -NMR indices (Table 7), the A/OA (alkyl C / *O*-alkyl C) ratio is generally taken as a sensitivity index for characterizing the decomposing extent of SOM (Baldock et al., 1997). When the value of the A/OA ratio is relatively high, it indicates that the degree of decomposition of SOM is high. In general, alkyl C and *O*-alkyl C keep a trade-off relationship (Li et al., 2013). The higher A/OA ratio in M1/2 and M1 could be because SOM in M1/2 and M1 was difficult to decompose further (Zhao et al., 2012). Therefore, moderate mowing (M1/2 and M1) enhanced the accumulation of stable fractions of SOM and recalcitrant chemical structures of SOC, and primed the degradation of labile C, which suggested that moderate mowing benefited the C stable sequestration in the semiarid grassland, which was significant to the grassland C pool. This foundation was reported for the first time in the grassland ecosystem. The carbohydrate C / methoxyl C (CC / MC) ratio is a new indicator to reflect the degree of degradation of SOM (Mathers et al., 2007), and both CC / MC and A / OA ratios showed that the degradation degree of SOM in M1 was the maximum. In addition to the highest CaHA content and highest herbage productivity in M1, M1 was the superior mowing management practice. The aliphaticity / aromaticity (Al / Ar) ratio is a predictor to reveal the complexity of the chemical composition of SOM, and the higher the value, the simpler the chemical composition of SOC. The hydrophobic C / hydrophilic C (HB / HI) ratio was used as a measure of C chemical recalcitrance, and the higher this value, the more stable the SOM (Boeni et al., 2014). The increased HB / HI ratios in M1/2 and M1 treatments showed that SOM was more recalcitrant to being mineralized. Meanwhile, the Al / Ar ratio revealed that M1/2 and M1 increased the chemical composition complexity of SOM, while M2 had no effect on either the chemical recalcitrance or complexity of SOM. These further proved that M1/2 and M1 improved the stability of SOM. The higher alkyl C in M1/2 and M1 is closely associated with the increase in recalcitrant compounds (waxes, resin, cutin, suberin, peptide side-chain, long-chain aliphatic

ics) (Table S1), mainly derived from the increased plant materials (Socher et al., 2012; Mariotte et al., 2013), accompanied by the loss of labile C such as carbohydrates and polysaccharides, and by the increase in lignin and cellular residues of microbes (Table 4). It is interesting that lignin C in treatment M2 was significantly higher than that in other treatments (Table 6), which might be because M2 limited the growth of degraders. In the future, it will be necessary to study the changes in functional microbial community in different mowing treatments using high throughput sequencing. Different from M1/2, M1 significantly increased *N*-alkyl/methoxyl C, which was recalcitrant C, and it was relatively enriched in topsoil when *O*-alkyl or di-*O*-alkyl C was prone to oxidation. In terms of the stability of SOM, M1 was the optimized mowing management practice.

4.3 Relationship among net N mineralization, microbes and chemical compositions of SOM

In natural grasslands, SOM mainly comes from plant litter, roots and soil microbial cellular residues, ca. 2–15 % of which was constituted by the N-containing compounds, such as amino acids, amino sugar, pyrimidines, purines or porphyrin (Mathers et al., 2007). Therefore, close correlations among the four C functional groups, net N mineralization and MBC were also observed, and MBC was significantly related to both C functional groups and net N mineralization (Tables 8 and 9), which suggested that microorganisms were the driving force of soil C and N turnover in the semiarid natural grassland. Li et al. (2017) reported that fungi might have played a more important role than bacteria in the N mineralization in the semiarid Inner Mongolia grassland, as fungi could better bear the drought and poor available nutrient conditions (Andresen et al., 2014; Mariotte et al., 2015). We also found that the correlation between C functional groups and MBC was consistent with that between C functional groups and net N mineralization. Stevenson et al. (2016) concluded that soils relatively rich in N should also be relatively rich in alkyl C, and the chemical composition of SOM significantly influenced soil N mineralization.

Similar to forest soil, recalcitrant C (alkyl C and aromatic C) also accounted for a large proportion of the SOC in the grassland soil. In our study, alkyl and aromatic C accounted for 40.9–47.1 % of all functional C. It was reported that fungi played the key role in the decomposition of soil organic N in the forest ecosystem (Boeni et al., 2014; Li et al., 2013), which indicated that fungi might also be critically important for the degradation of organic N in the grassland ecosystem. Li et al. (2017) reported that mowing once a year increased fungal abundance and diversity, while a higher mowing frequency decreased them. The increased fungal communities were characterized by the function of mineralizing SOM and activating nutrients. In the semiarid grassland, the contents of soil rapidly available N and available P were very low, and mycorrhizal fungi were richer in M1. Northup et

al. (1998) found a mechanism by which plant productivity could be sustained through mycorrhizal fungi by investigating plant–soil–microbe interaction. M1/2 and M1 improved herbage productivity and thus increased net SOC content mainly by increasing recalcitrant C, and further increased microbial community diversity and dominant microbial community abundance. In turn, the increased microbial community enhanced the labile SOM degradation and the humification of SOM to make the chemical composition of SOM more stable, and this agreed with the studies conducted by Baumann et al. (2013) and Zhang et al. (2015). Thus, the relationship between chemical composition of SOM, SOM mineralization and the microbial community would give us a better understanding of the stability of soil C and N pools.

5 Conclusions

Long-term M1/2 and M1 treatments significantly enhanced the accumulation of SOM by increasing the CaHA content and lignin, while the higher-frequency mowing practice (M2) limited the accumulation of SOM. Mowing had significant impacts on the fractions and chemical structure of SOM. M1/2 and M1 significantly increased soil CaHA and MBC content and improved the stability of SOM by increasing alkyl C and aromatic C functional groups, which suggested that the humification was enhanced, while higher-frequency mowing practice (M2) had a negative impact on the stability of SOM. Therefore, M1/2 and M1 were considered moderate mowing practices, while M2 should be avoided from a long-term perspective. M1 was the best mowing practice because it increased the stability of SOM by elevating a stable chemical structure of SOM and enhancing the humification of SOM. In addition, the ^{13}C -NMR indices could consistently reflect the stability of SOM. The impacts of mowing on the accumulation and stability of SOM were closely related to soil microbes and SON mineralization. Moderate mowing managements were beneficial for more microbes to degrade the labile SOM to provide N for plant growth, and this increased the SOM input in turn. Solid CPMAS ^{13}C -NMR is a powerful technique assessing the complex samples, e.g. soil, and it showed that alkyl C and *O*-alkyl C were the dominant chemical components of grassland SOC under different mowing treatments, followed by aromatic C and carbonyl C. However, to better understand the biological mechanisms of SOM chemical shifts resulting from different mowing managements, it is necessary to further investigate the microbial community diversity and the relationship between the C functional groups and microbial community diversity by combining advanced NMR and high-throughput sequencing techniques.

Data availability. Data for this paper can be found in the Supplement.

The Supplement related to this article is available online at doi:10.5194/bg-14-2685-2017-supplement.

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

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