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

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**Abstract.** Grassland is the second largest carbon pool following forest. 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 was 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 $^{13}$C-NMR, CPMAS $^{13}$C-NMR) and Fourier Transformed 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 group (*O*-alkyl and carbonyl C) and forming recalcitrant humus while M2 had opposite effects. The consistent increase of the values of NMR indices reflecting the degradation degree, hydrophobicity and aromaticity of SOM in M1 reflected that M1 had the largest contribution in increasing the stability of SOM while these values in M2 were similar to those in M0. Significant correlations between different SOM fractions 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 of SOM fractions and chemical composition were closely related to soil microbial biomass and activity. Therefore, in the view of quality and quantity of SOM and the sustainable development of grassland ecosystem, M1 was the optimal mowing management while M2 should be avoided in the semiarid grassland.

**Key words:** Mowing, Soil organic matter composition, Solid state CPMAS $^{13}$C-NMR, grassland

# 1 Introduction

Soil organic matter (SOM) plays a central role in the global biogeochemical cycles of most major nutrients. Soil C pool is the largest C storage in the terrestrial ecosystem and the organic C pool consists of more than 90% of 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, represents more than a quarter of the total grassland area (China's Environmental Bulletin, 2006). Therefore, soil C and nitrogen (N) cycling in Inner Mongolia grassland have been a hot topic (Shan et al., 2011, Wang et al., 2014; Wang et al., 2016). Mowing once a year is one of the common practice in grassland ecosystem 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 were 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 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) included simple but resistant C like alkyl C, a decomposition product which is stable in soil. The stability of 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 pool. 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, liable SOC fractions include water soluble organic C (WSOC), microbial biomass C (MBC) and readily oxidization 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., 2012; Zhou et al., 2014). The application of advanced solid-state NMR, i.e. cross-polarization magic angle spinning $^{13}$C-NMR (CPMAS $^{13}$C-NMR) in characterizing chemical structures of SOM, is also an important approach to reveal the essential changes of SOM formation and degradation in paddy field and forest ecosystems (Zhou et al., 2014; Panettieri et al., 2014; Zhang et al., 2015) and in litter and wood decomposition processes in these ecosystem (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). These 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), 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 included in lignin while *O*-alkyl and di-*O*-alkyl C are included in polysaccharide (carbonhydrates) (Preston et al., 1998; Bonanomi et al., 2013). By analyzing 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., 2015). 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′N – 44°089′N and 116°049′E – 117°059′E) in northern China. It had 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 evaportranspiration ranged from 1,600 to 1,800 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 et al., 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 the 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 started 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 3 replicates. The four treatments were unmown (M0), mowing once every two years (M1/2) in Aug., mowing once a year (M1) in Aug. because the peak aboveground biomass of *L. chinensis* was in Aug. and mowing twice every year (M2) in Jun. when the palatability of *L. chinensis* was best for the livestock and in Sep. when *L. chinensis* was withered.

### 2.2 Soil samples collection

Soil samples were collected from 0 – 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 two parts. One part was air-dried for basic physical and chemical properties analysis, the other fresh part was used to analyze 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-hydrolyzable N (AN), Olsen phosphorus (Olsen P) and net N mineralization were 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 TOC analyzer (Elementar Liqui TOC, Elementar Co., Hanau, Germany). Water soluble organic carbon (WSOC) was determined using a modified method (Li et al., 2013). Briefly, WSOC were 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 ×g, 20 min), and then the supernatant was filtered using a 0.45 μm membrane filter. The filtrate was measured by the same TOC analyzer 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 gram 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 them, 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 pretreated 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) and the C and N content in 10% HF-treated SOM samples and bulk soil samples were 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, America). Each sample was prepared by grinding 2 mg freezing-drying SOM sample with 200 mg oven-dried KBr in a vibrating puck mill and then about 150 mg mixtures were compressed into a translucent pellet using a hydraulic compressor. The pellet was immediately placed on the sample holder, and all spectra ranging from 4,000 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 4,000 to 1,000 $cm^{-1}$ of FTIR spectra were assigned because peaks in the fingerprint region below 1,000 $cm^{-1}$ were difficult to assign and were very complex, usually overlapping with signals of inorganic soil minerals.

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

Solid-state $^{13}$C-NMR experiment was performed on a Bruker Avance II 300 (Bruker Instrumental Inc) equipped with a 7 mm CPMAS (cross-polaration magic-angle-spinning) detector. NMR spectra were acquired under the conditions of a spectrometer frequency of 75 MHz, a MAS spinning frequency of 5,000 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 was statistically analyzed 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 was the mean values (n = 3). Linear regression analysis was conducted after the Pearson product-moment correlation analysis by 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 treatment 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 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. 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 – 0.63% and 0.25 – 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 ratio 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 ratio 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 was shown in Fig. 1. The spectra were dominated by the broad peak around 3,406 cm$^{-1}$, sharp peaks around 1,030 cm$^{-1}$ and medium sharp peaks around 1,653 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 2,928 and closing to 1,500 cm$^{-1}$ due to aliphatic C-H stretching in CH$_2$/CH$_3$ and amide N-C/amino-NH vibrations, and aliphatic C-H bending in CH$_2$/CH$_3$, respectively, were found in all treatments. However, only some small differences in the intensity of the peaks sowed in the FTIR spectra were showed qualitatively between different treatments. The intensity of the peak at 2,928 cm$^{-1}$ in M1 was stronger than that in M0 and M2 while the intensity of the peak at 1,030 cm$^{-1}$ in M1 was weaker than that in M0 and M2 (Fig.1 and Table 3).

Fig. 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 were medium. Compared to M0, mowing significantly increased alkyl C but significantly decreased substituted alkyl C (except for $N$-alkyl/methoxyl 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 was 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 indexes reflecting soil quality directly or indirectly were calculated and the results were shown in Table 6. Soil lignin C, A/OA ratio and HB/HI ratio in M0 were significantly lower than those in the mowing treatments ($P$ <0.05), while 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 opposite ($P$ <0.05) which resulted in Al/Ar ratio in M1/2 and M1 was significant higher than that in M0. There was no difference in aliphaticity, aromaticity and Al/Ar ratio between M0 and M2 ($P$ >0.05). Among different mowing treatments, most of $^3$C-NMR indexes in M1/2 are similar to M1 except that aromaticity in M1/2 was significantly lower than that in M1 while that aliphaticity and CC/MC ratio in M1/2 was significantly higher than those in M1 ($P$ <0.05). In all of the indexes, lignin C, L/P ratio and aliphaticity in M2 were significantly higher than those in both M1/2 and M1 and the other indexes was on the contrary ($P$ <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, 0.54 ($P$ <0.05), but 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 – 0.90, $P$ <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, CaHA with $r$ = 0.60, 0.83, 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 five 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 to *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 r, 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 to 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 not correlated with *N*-alkyl/methoxyl C and *O*-alkyl C.

## 4  Discussion

### 4.1 SOM accumulation impacted by different mowing practices for a long term

Our results showed that 12-years M1/2 and M1 treatments 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 (Marriotte et al. 2015; Socher et al., 2012). Enhanced plant species richness promoted plant productivity and photosynthesis, 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 include 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 of CaHA content in M1/2 and M1 was the main and direct reason of 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 of 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 of 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 atom H/C ratio indicated more aromatic compounds or higher aromaticity

and saturability, and the higher atom O/C ratio indicated more carboxyl groups, phenol or carbohydrates with oxygen (Ma et al., 2001; Steelink et al., 1985; Kim et al., 1991). ^{13}C NMR apparently differentiated the lignin C (include alkyl, *N*-alkyl and aryl C) and carbohydrate C (include *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 which 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 included carbonyl and carboxyl C was relatively easy to be degraded.

The accumulation of lignin and the increase of microbial biomass were the favorable conditions of humification. Therefore, CaHA fraction in SOM increased by 46.9 – 52.5% after 12 years. These suggested that long-term moderate mowing managements enhanced the degree of humification of SOM. On the contrary, 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 human excessive activity resulted in the humic horizons disappearance in many tropic regions. These 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 functions (Steinauer et al., 2015).

In the CPMAS ^{13}C-NMR indices (Table 7), A/OA (alkyl C/*O*-alkyl C) ratio is generally taken as a sensitive index of the decomposition extent of SOM (Baldock et al., 1997). When the value of 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 considered that SOM in M1/2 and M1 treatments was difficult to be further decomposed (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 as well as 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. The carbonhydrate C/methoxyl C (CC/MC) ratio is a new indicator to reflect the degree of degradation of SOM (Mather et al., 2007), and both CC/MC and A/OA ratios showed the degradation degree of SOM in M1 treatment was the maximum. In addition to the highest CaHA content and highest herbage productivity in M1 treatment, 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 manifested that SOM was more recalcitrant to be mineralized. Meanwhile, Al/Ar ratio revealed that M1/2 and M1 increased the chemical composition complexity of SOM while M2 had no effect on both the chemical recalcitrance and 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 of recalcitrant compounds (waxes, resin, cutin, suberin, peptide side-chain, long-chain aliphatics) (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, polysaccharides, and by the increase of 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 is necessary to study the changes of functional microbial community in different mowing treatments using high throughput sequencimg. 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 stability of SOM, M1 was the optimized mowing management practices.

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

In natural grasslands, SOM mainly come from plant litter, roots and soil microbial cellular residues, and 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 (Table 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 played a more important role 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). Our 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 higher mowing frequency decreased them. The increased fungal communities characterized with the function of mineralizing SOM and activating nutrients (Li et al., 2107). In the semiarid grassland, the contents of soil rapidly available N and available P was very low and mycorrhizal fungi was richer in M1 treatment. Northup et al., (1998) found a mechanism by which plant productivity could be sustained was through with mycorrhizal fungi though investigating plant-soil-microbe interaction. M1/2 and M1 improved herbage productivity and thus net increased 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 agreeed with the studies conducted by Baumann et al., (2013) and Zhang et al., (2015). Thus, the relationship between chemical composition of SOM, SOM mieralization and 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, 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 stable chemical structure of SOM and enhanced 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-through sequence techniques.

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**Figure captions:**

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

**Fig. 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.
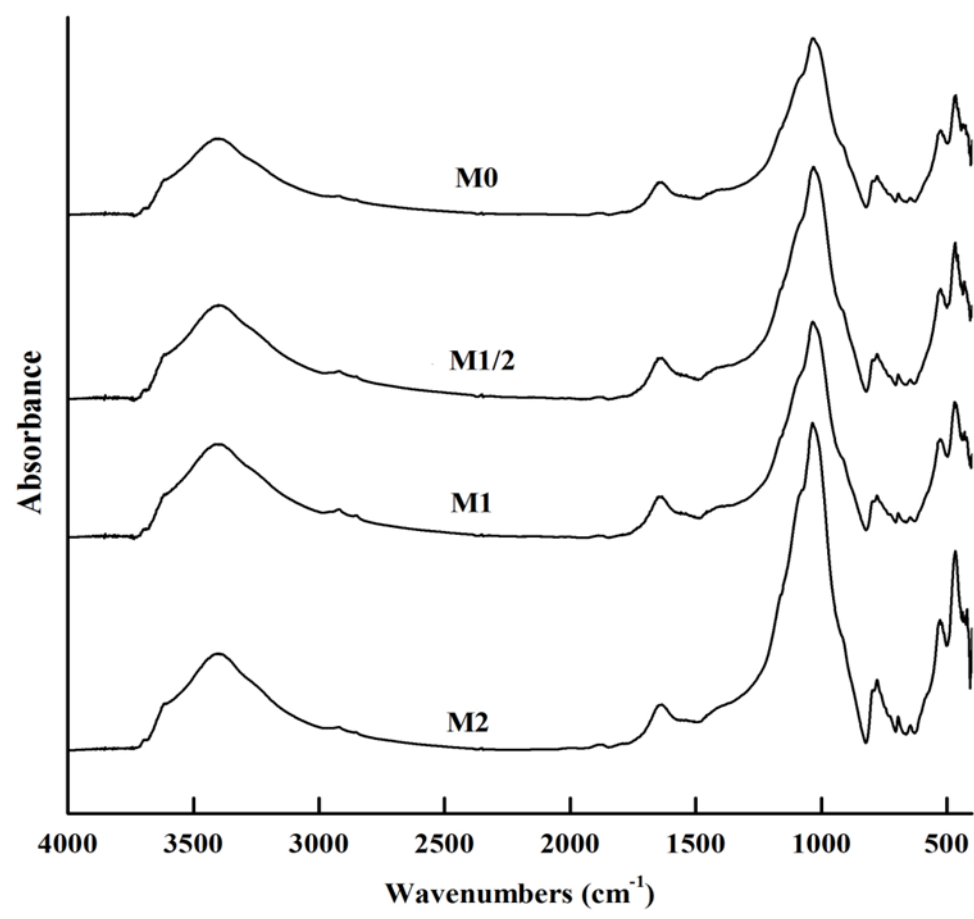
Fig. 1 FTIR spectra of 10% HF pretreated SOM samples under long-term different mowing managements.
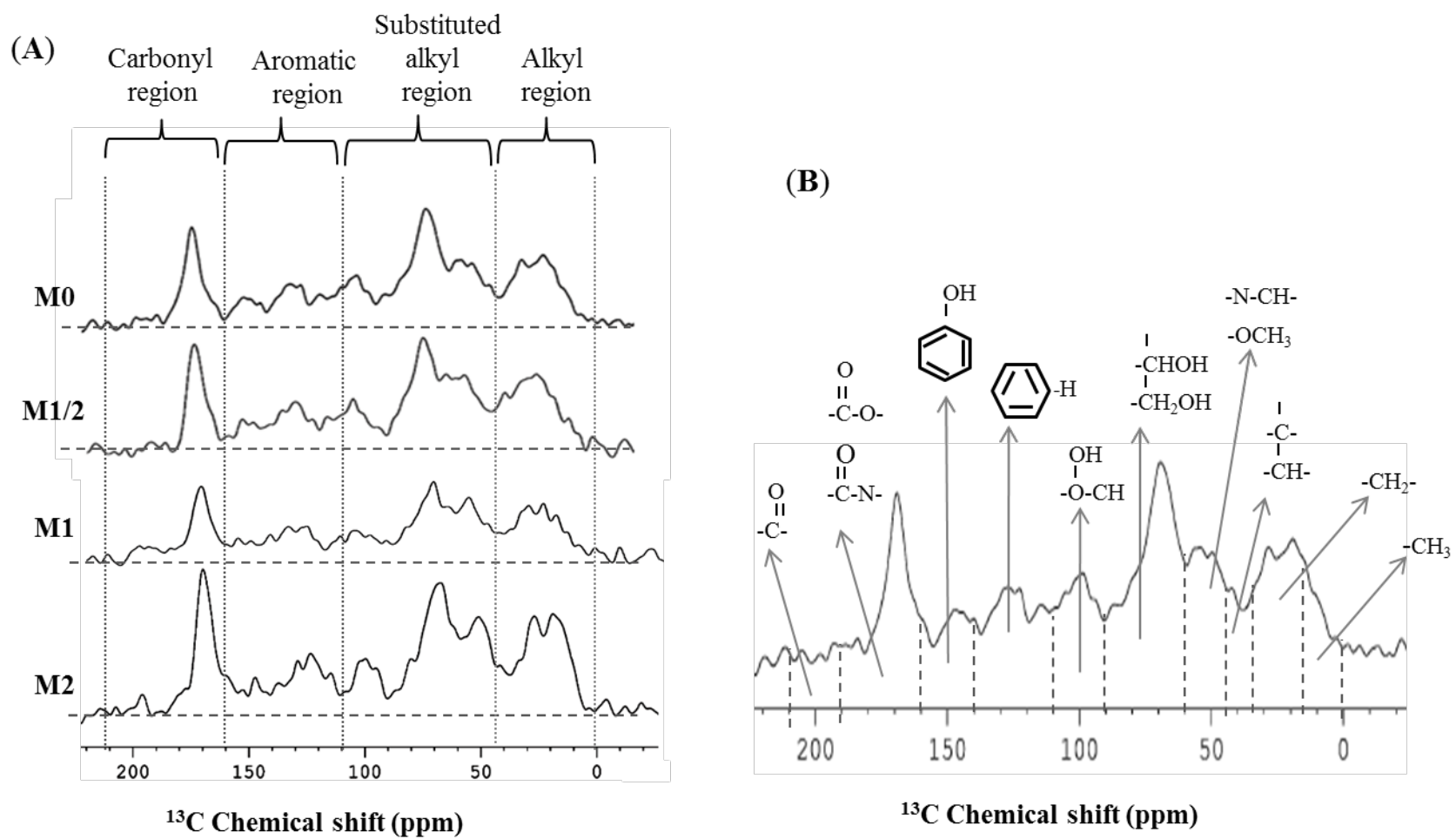
Fig. 2 CPMAS $^{13}$C-NMR spectra of 10% HF pretreated SOM. **A**, CPMAS $^{13}$C-NMR spectra of 10% HF pretreated SOM under long-term different mowing managements. **B**, detail C functional groups in different chemical shifts.

**The list of Tables**

**Table 1** Calculation formulas of different $^{13}$C-NMR indexes

**Table 2** Basic description of soil properties under different mowing intensities

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

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

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

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

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

**Table 8** Summary of the linear correlations between Net N mineralization, MBC and all the C functional groups of SOC determined by CPMAS $^{13}$C-NMR

**Table1**

Calculation formulas of different $^{13}$C-NMR indexes

| Index | Formula | Reference |
|---|---|---|
| A/OA | alkyl C (45 – 0 ppm) / O-alkyl C (110 – 60 ppm) | Maters et al., (2007) |
| CC/MC | carbonhydrate 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) | |
| Aromaticity, % | aromatic C *100 / ( alkyl C + substituted C + aromatic C) | Zhao et al., (2012) |
| Al/Ar | aliphaticity / aromaticity | |
| Lignin C | phenolic C *4.5 + methoxyl C | |
| Polysaccharide C | 1.2*(O-alkyl C – phenolic C *1.5) | Preston et al., (1998) |
| L/P | lignin C/ polysaccharide C | |

**Table 2**

Basic description of soil properties under different mowing treatments

| Treatment | pH H$_2$O | SOC g kg$^{-1}$ | TN | Olsen P mg kg$^{-1}$ | AN | Net N mineralization 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 the difference between treatments reaches 5% significant 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 oxidization carbon. MHA, mobile humic acid. CaHA, calcium humic acid. SOM, soil total organic matter. HA = MHA+CaHA.

**Table 3**

| Treatment | MBC | WSOC | ROC | MHA | CaHA | HA/SOM |
|---|---|---|---|---|---|---|
| | mg kg$^{-1}$ | | | g kg$^{-1}$ | | % |

**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.

| 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 |

**Table 5**

Percentages of total special spectral area 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 | 60 – 45 ppm | 90 – 60 ppm | 110 – 90 ppm | 140 – 110 ppm | 165 – 140 ppm | 210 – 165 ppm |
| | Alkyl | *N*-alkyl/methoxyl | *O*-alkyl | di-*O*-alkyl | Aryl | *O*-aryl | 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 | Lingin-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 = carbonhydrate C/methoxyl C. Al/Ar, Aliphaticity/Aromaticity.

**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** |
| *N*-alkyl/methoxyl C (60 – 45) | 0.28 | 0.615 | 0.29 | 0.891 |
| *O*-alkyl C (90 – 60) | 0.37 | 0.429 | 0.27 | 0.992 |
| di-*O*-alkyl C (110 – 90) | 0.47 | 0.326 | 0.59 | **0.027** |
| Aryl C (140 – 110) | -0.24 | 0.798 | 0.73 | **0.011** |
| *O*-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 to *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.