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Nitrogen deposition impacts on the amount and stability of soil organic matter in an alpine meadow ecosystem depend on the form and rate of applied nitrogen

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Summary

The effects of atmospheric nitrogen (N) deposition on carbon (C) sequestration in terrestrial ecosystems are controversial. Therefore, it is important to evaluate accurately the effects of applied N levels and forms on the amount and stability of soil organic carbon (SOC) in terrestrial ecosystems. In this study, a multi-form, small-input N addition experiment was conducted at the Haibei Alpine Meadow Ecosystem Research Station from 2007 to 2011. Three N fertilizers, NH_4Cl, $(NH_4)_2SO_4$ and KNO_3, were applied at four rates: 0, 10, 20 and 40 kg N ha^{-1} year^{-1}. One hundred and eight soil samples were collected at 10-cm intervals to a depth of 30 cm in 2011. Contents and δ^{13}C values of bulk SOC were measured, as well as three particle-size fractions: macroparticulate organic C (MacroPOC, > 250 µm), microparticulate organic C (MicroPOC, 53–250 µm) and mineral-associated organic C (MAOC, < 53 µm). The results show that 5 years of N addition changed SOC contents, δ^{13}C values of the bulk soils and various particle-size fractions in the surface 10-cm layer, and that they were dependent on the amounts and forms of N application. Ammonium-N addition had more significant effects on SOC content than nitrate-N addition. For the entire soil profile, small additions of N increased SOC stock by 4.5% (0.43 kg C m^{-2}), while medium and large inputs of N decreased SOC stock by 5.4% (0.52 kg C m^{-2}) and 8.8% (0.85 kg C m^{-2}), respectively. The critical load of N deposition appears to be about 20 kg N ha^{-1} year^{-1}. The newly formed C in the small-input N treatment remained mostly in the > 250 µm soil MacroPOC, and the C lost in the medium or large N treatments was from the > 53 µm POC fraction. Five years of ammonium-N addition increased significantly the surface soil POC:MAOC ratio and increased the instability of soil organic matter (SOM). These results suggest that exogenous N input within the critical load level will benefit C sequestration in the alpine meadow soils on the Qinghai–Tibetan Plateau over the short term.

Introduction

Currently, the spatial distribution, pathways and driving mechanisms of the missing carbon (C) sink are important issues in global C cycle research. Increasing atmospheric nitrogen (N) deposition can significantly change C cycling rates and the C budget in terrestrial ecosystems, and is generally considered to be an important pathway contributing to the missing sink (Magnani *et al.*, 2007). The effects of atmospheric N deposition on C sequestration in terrestrial ecosystems are variable. The effects include increased C sequestration (Magnani *et al.*, 2007), promoted C loss (Bragazza *et al.*, 2006) and no change (Nadelhoffer *et al.*, 1999). Overall, it is most likely that atmospheric N deposition promotes C sequestration (Janssens *et al.*, 2010). However, the efficiency of estimated C sequestration in terrestrial ecosystems caused by N deposition varies, ranging from 60 to 200 kg C kg^{-1} N (Högberg, 2007; Magnani *et al.*, 2007). It is apparent that there is considerable uncertainty about how C dynamics and C sequestration in terrestrial ecosystems respond to increased N deposition. Therefore, more detailed studies in various ecosystems are needed to reduce these uncertainties.

The response of below-ground C processes to N deposition is more complicated than that of above-ground processes. Similarly, the biogeochemical effects of N deposition input or N fertilizer application on SOC storage are also complicated (Lu *et al.*, 2011). Nitrogen addition can increase, decrease or have no effect on SOC

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contents (Neff *et al.*, 2002; Liu & Greaver, 2010; Lu *et al.*, 2011). Labile fractions of SOC are more sensitive indicators of N addition than total SOC (Heitkamp *et al.*, 2011). Separating SOC on a particle-size basis into various functional pools may help to identify the mechanisms behind observed effects of elevated N inputs on soil C dynamics. It is necessary to explore the accumulation and loss of SOC fractions with different particle sizes to predict SOC dynamics under various applied N rates and forms. Although the effects of N addition on contents of SOC and its fractions have been well documented for agricultural soils (Manna *et al.*, 2006), there is little information for natural alpine meadow ecosystems (Neff *et al.*, 2002).

The ^{13}C natural abundance (or $\delta^{13}C$) technique provides a way to characterize the dynamics of SOC with different turnover times (Balesdent *et al.*, 1996). In general, coarse-sized and light-density fractions of soil organic matter (SOM) originated from recent plant residues and underwent a short-term decomposition. In contrast, fine-sized and heavy-density SOM originated from old plant materials and experienced a long-term decomposition (Cadisch *et al.*, 1996). In the C_3-vegetation-dominated ecosystem, coarse-sized SOC fractions often represent litter-derived ^{13}C-depleted signals, whereas fine-sized SOC fractions associated with clay and silt maintain the ^{13}C-enriched signals (Fang *et al.*, 2009). Thus, our hypothesis is that N addition increases SOC content and tends to decrease $\delta^{13}C$ through an increase in litter-derived and ^{13}C-depleted coarse-sized SOC. On the other hand, N addition can also decrease SOC content and increase $\delta^{13}C$ through accelerating the emission of ^{13}C-depleted CO_2.

The Qinghai-Tibetan Plateau covers about 2.5 million km^2 and alpine meadows occupy about 35% of this area (Yang *et al.*, 2008). Soil C storage and SOC density at 1-m depth have been estimated to be 4.68 Pg C and 9.05 kg m^{-2}, respectively; soil C storage accounted for about one twentieth of total soil C storage in China in the 1980s (Yang *et al.*, 2008). The alpine meadow is a typical ecosystem limited by N and is potentially sensitive to an increase in N availability. Atmospheric N deposition of the Qinghai-Tibetan Plateau has rapidly increased over the past two decades (Lü & Tian, 2007), and this will influence the C cycle and C sequestration in the alpine meadow ecosystem. Previous studies have shown that a small N input can significantly change the interaction between plant and soil microbes, and further increase soil N availability and CO_2 emission in the N-limited ecosystem (Fang *et al.*, 2012).The objectives of this study were to (i) determine the effects of the form and rate of N addition on the contents and $\delta^{13}C$ values of organic C in bulk soil and different sized particles, (ii) quantify the net changes of SOC stock and stability caused by N addition and (iii) evaluate the relative contributions of different SOC fractions to variations of SOC content and stability under N addition.

Materials and methods

Study site

This study was conducted at the Haibei Alpine Meadow Ecosystem Research Station, Chinese Academy of Sciences (37°37′N, 101°19′E, 3220 m above sea level), which is located on the eastern edge of the Qinghai-Tibetan Plateau. The region is characterized by a typical plateau continental climate. The mean annual temperature is −1.7°C, with a monthly mean temperature ranging from −14.8°C in January to 9.8°C in July (Fang *et al.*, 2012). The mean annual precipitation ranges from 426 to 860 mm, of which more than 80% occurs between May and September (Fang *et al.*, 2012). The vegetation is typical of the *Kobresia humilis* meadow. Dominant species belong to perennial herbs, and can be divided into four functional groups: grasses (*Festuca ovina* L., *Festuca rubra* L., *Stipa aliena* Keng, *Elymus nutans* Griseb, *Helictotrichon tibeticum* (Roshev.) Holub, *Koeleria cristata* (L.) Pers.), sedges (*Kobresia humilis* (C. A. Mey ex Trauvt.) Sergievskaya, *Poa crymophila* Keng), legumes (*Leontopodium nanum* (Hook. f. et Thoms.) Hand. Mazz) and forbs (*Saussurea superb* Anthony, *Potentilla saundersiana* Royle, *Lancea tibetica* Hook. f. et Thoms). The study area is subjected to light grazing and the vegetation coverage is more than 90% (Fang *et al.*, 2012). The soils developed in this ecosystem are Cambisols.

Experimental design

The N addition experiment was a split-plot design with the different N rates making up the main plots and the N forms as subplots. To simulate deposition of reduced NH_4^+ and oxidized NO_3^-, three N fertilizers (NH_4Cl, $(NH_4)_2SO_4$ and KNO_3) were used. The treatments of added N were, in relation to the local atmospheric N deposition at the Haibei station (8.7–13.8 kg N ha^{-1} $year^{-1}$), defined as control (0 kg N ha^{-1} $year^{-1}$), small (10 kg N ha^{-1} $year^{-1}$), medium (20 kg N ha^{-1} $year^{-1}$) and large (40 kg N ha^{-1} $year^{-1}$) rates and were used to simulate a future increase in the atmospheric N deposition by one, two and fourfold. Each N treatment was replicated three times, so the experiment includes a total of 36 subplots. Each subplot was 9 m^2 in area (3 × 3 m^2), and subplots were separated by 2-m wide buffer strips. Nitrogen fertilizer solutions were sprayed on to the subplots with a sprayer during the first week of each month over the entire year, while the equivalent amount of water was sprayed onto the control subplots. This experiment was conducted over 5 years beginning in May 2007 and ending in September 2011 (Fang *et al.*, 2012).

Soil sampling and processing

In September 2011, five soil cores were collected from each subplot to a depth of 30 cm at intervals of 10 cm with an auger (2.5 cm in diameter). The average thickness of the humus layer (A layer) was about 10 cm. Soil samples were immediately passed through a 2-mm sieve to remove roots, gravel and stones, and then stored in resealable plastic bags. To assess the effects of N addition, we assumed that the SOC contents in the control subplots were stable over the entire period of the experiment.

A wet-sieving procedure adapted from Cambardella & Elliott (1992) was used to separate the bulk soil into three particle-size fractions. Simply, 50 g air-dried soil samples and 100 ml 1% sodium hexametaphosphate solution were placed in polycarbonate bottles and shaken overnight at a rate of 200 rpm min^{-1}. Macroparticulate organic C (MacroPOC, > 250 μm) and microparticulate organic C (MicroPOC, 53–250 μm) were recovered by back-washing the sieve followed by filtration (Whatman filter paper #541). Particulate OC is the sum of MacroPOC and MicroPOC. The mineral-associated organic C (MAOC, < 53 μm) fraction was recovered by evaporation. All three fractions were dried overnight at 60°C and weighed.

Chemical and isotopic analysis

Organic C contents (g C kg^{-1} soil) in bulk soil and soil particle samples were determined by dry combustion using a CHN auto-analyser (Flash EA1112, Thermofinnigan, Milan, Italy). Stable C isotope compositions ($\delta^{13}C$) in bulk soil and soil particle samples were determined with an automated continuous-flow isotope ratio mass spectrometer (Finnigan MAT-253, Thermo Electron, Bremen, Germany). Soil organic C stock was calculated by multiplying the SOC content (g kg^{-1}) by the soil thickness (cm), the corresponding bulk density (g cm^{-3}) and the proportion of a given size particle in the soil < 2 mm (Yang *et al.*, 2008). During the 5 years of N addition, we measured soil bulk density only once by using the cutting ring method in September 2011. Therefore, we assumed that there was no change of soil bulk density in each subplot.

Statistical analysis

We used a nested analysis of variance (ANOVA) to test the resulting differences in C contents and $\delta^{13}C$ values of bulk soil and three particle-size fractions. For each N fertilizer type, we used one-way ANOVA combined with the Fisher's least significant difference (LSD) test to compare the difference in the means. The results are provided in the Supporting information (Tables S1, S2). All statistical analyses were conducted using the SPSS software package (version 16.0), and statistically significant differences were set with P values at < 0.05 unless otherwise stated.

Results

SOC content and $\delta^{13}C$ value in bulk soil

In the control, the total SOC contents and $\delta^{13}C$ values at the 0–10-cm depth averaged 48.0 g kg^{-1} and −25.75‰, respectively (Figure 1). Five years of N addition changed SOC contents at the 0–10-cm depth only (Table 1). The N rate rather than the form significantly changed SOC contents at the 0–10-cm depth (Table 1, $P = 0.003$). For ammonium-N fertilizer treatments (NH_4Cl and $(NH_4)_2SO_4$), the small N input tended to increase SOC contents in the 0–10-cm layer, whereas medium N tended to decrease SOC contents in the same layer (Figure 1a,b). The difference in SOC contents between the control and medium N treatments was marginally significant (Table S1). However, applied KNO_3 fertilizer did not significantly change SOC contents in any of the soil layers (Table 1, Table S1).

The response of soil $\delta^{13}C$ value to N addition was consistent with SOC content. Nitrogen rates rather than N forms marginally changed soil $\delta^{13}C$ values in the 0–10-cm layer (Table 1, $P = 0.093$). A small rate of applied ammonium-N fertilizers tended to deplete soil $\delta^{13}C$ values, whereas medium or large ammonium-N rates tended to enrich soil $\delta^{13}C$ values (Figure 1d,e). The difference in soil $\delta^{13}C$ values between medium $(NH_4)_2SO_4$ addition and control was significant (Figure 1e, Table S1). However, applied KNO_3 fertilizer did not significantly influence soil $\delta^{13}C$ values (Table S1). These results indicate that applied ammonium-N fertilizer had larger effects on SOC contents and $\delta^{13}C$ values in bulk soils than applied nitrate-N fertilizer.

SOC contents and $\delta^{13}C$ values in various particle-size fractions

In the control, the means of soil MacroPOC, MicroPOC and MAOC contents in the 0–10-cm layer were 13.6, 18.8 and 15.6 g C kg^{-1} soil, respectively. Soil POC accounted for 67.1% of total SOC (Figures 2–4). Nitrogen rate significantly changed the SOC contents of the three particle-size fractions in the top 10 cm (Table 1, $P = 0.007$, $P = 0.047$, $P = 0.027$ for MacroPOC, MicroPOC, MAOC, respectively); however, N forms significantly changed the soil MAOC contents only (Table 1, $P = 0.003$). For each N fertilizer type, small rates of NH_4Cl and $(NH_4)_2SO_4$ fertilizers tended to increase soil MacroPOC contents in the top 10 cm layer, whereas an opposite response was found in the large rate of NH_4Cl and medium rate of $(NH_4)_2SO_4$ treatments (Figure 2a,b). Inconsistently, a medium rate of KNO_3 addition still tended to increase soil MacroPOC contents (Figure 2c, Table S1). Except for the medium rate of $(NH_4)_2SO_4$ treatment, N addition did not change significantly the soil MicroPOC contents (Figure 3a,c, Table S1). The soil MAOC content was also sensitive to N addition. Small and medium rates of applied ammonium-N fertilizers increased significantly the soil MAOC contents, while opposite responses were found in the large N treatments (Figure 4a,b, Table S1). A large rate of applied KNO_3 increased significantly the soil MAOC contents (Figure 4c, Table S1).

The depletion or enrichment of $\delta^{13}C$ values of three particle-size SOC fractions caused by N addition depended on the rates and forms of applied N. In the control, the average $\delta^{13}C$ values of MacroPOC and MicroPOC in the 0–10-cm soil layer were −26.00 to −26.18‰, respectively, which was less than that of MAOC in the same soil layer (−24.99‰) (Figures 2–4). Both N rates and forms changed significantly the $\delta^{13}C$ values of MacroPOC and MAOC (Table 1, $P < 0.05$); furthermore, N forms marginally changed the $\delta^{13}C$ values of MicroPOC (Table 1, $P = 0.059$). For applied ammonium-N treatments, the small N rate did not change the $\delta^{13}C$ values of MacroPOC, MicroPOC and MAOC, while the medium rate of $(NH_4)_2SO_4$ and large rate of NH_4Cl enriched significantly the $\delta^{13}C$ values of three particle-size SOC fractions (Figures 2–4, Table S1). However, applied KNO_3 fertilizer did not change the $\delta^{13}C$ values of MacroPOC and MicroPOC, but medium N treatments significantly enriched the $\delta^{13}C$ values of MAOC (Figures 2–4, Table S1).

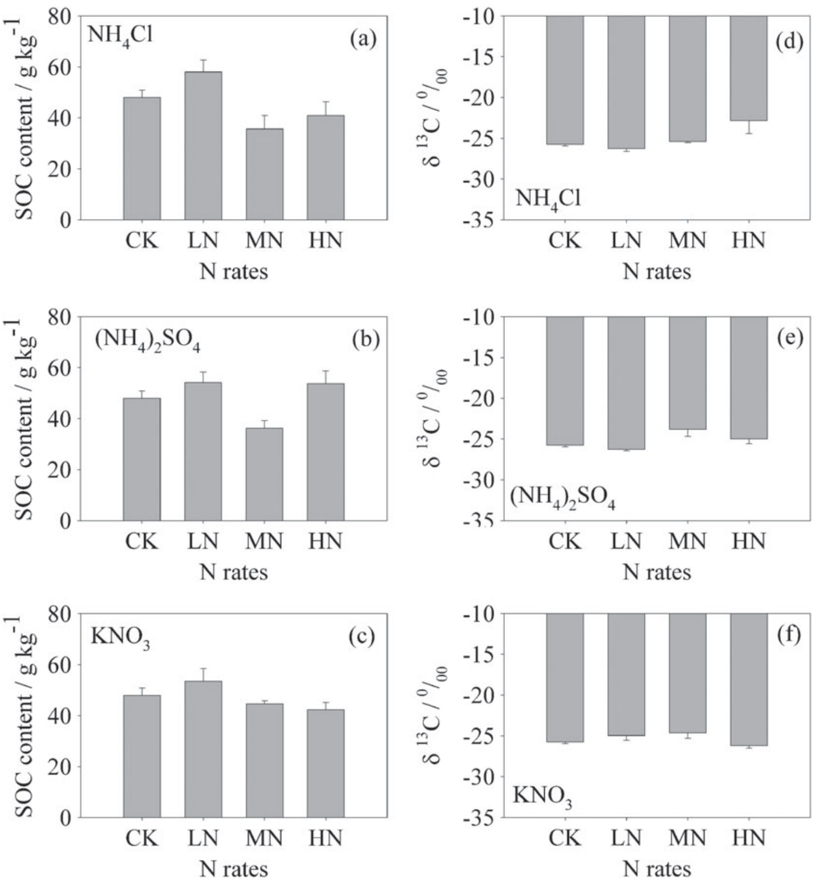

Figure 1 Total SOC contents and average δ^{13}C values in bulk soils in the 0–10-cm layer under different N rates and N forms (a–f). Soil samples were collected from the alpine meadow on the Qinghai-Tibetan Plateau in 2011. Values are means of three replicates ± standard error.

Table 1 *P*-values of nested ANOVA of the effects of applied N rates (10, 20 and 40 kg N ha^{-1} year^{-1}) and N forms (NH_4Cl, $(NH_4)_2SO_4$ and KNO_3) on SOC contents and δ^{13}C values in bulk soils and various particle-size fractions in three soil layers

		Bulk soil		MacroPOC[b]		MicroPOC[b]		MAOC[b]		
Soil horizon / cm	Source of variation[a]	SOC	δ^{13}C	SOC	δ^{13}C	SOC	δ^{13}C	SOC	δ^{13}C	POC/MOC
0–10	Rate	0.003	0.093	0.007	0.016	0.047	0.18	0.027	0.030	0.014
	Form (rate)	0.59	0.16	0.13	0.009	0.64	0.059	0.003	0.004	0.038
10–20	Rate	0.79	0.61	0.13	0.57	0.73	0.98	0.25	0.29	0.12
	Form (rate)	0.61	0.99	0.28	0.93	0.17	0.84	0.93	0.79	0.41
20–30	Rate	0.56	0.36	0.46	0.79	0.85	0.64	0.30	0.40	0.42
	Form (rate)	0.77	0.82	0.11	0.99	0.67	0.94	0.78	0.66	0.45

[a]Form (rate) described in the model means the N forms within N addition level.

[b]MacroPOC, MicroPOC and MAOC refer to macroparticulate organic C (> 250 μm), microparticulate organic C (53–250 μm) and mineral-associated organic C (< 53 μm), respectively.

Soil POC:MAOC ratio

Both N rates and forms changed significantly the soil POC:MAOC ratios at 0–10-cm depth (Table 1, $P = 0.014$, $P = 0.038$ for N rate and form, respectively). For two ammonium-N fertilizer treatments, small and medium rates of applied N did not change the soil POC:MAOC ratio, but the large rate significantly increased it (Figure 5a,b, Table S1). Although applied KNO_3 fertilizer tended to decrease soil POC:MAOC ratios, the difference between applied N treatments and control was not significant ($P > 0.05$) (Figure 5c, Table S1).

The net changes of SOC stocks and δ^{13}C values

For the whole soil profile, the small N rate tended to increase the SOC stock of bulk soils and > 53 μm POC fractions, whereas the opposite patterns were found at the medium and large N rates (Figure 6a). The increase in SOC stock caused by the small N rate was 0.43 kg C m^{-2} and mainly resulted from the increase in the MacroPOC fraction (Figure 6a, Table S2). The decreases in SOC stock caused by medium or large N rates were 0.52 and 0.85 kg C m^{-3}, respectively, originating from the MicroPOC and MacroPOC components (Figure 6a, Table S2). Simultaneously,

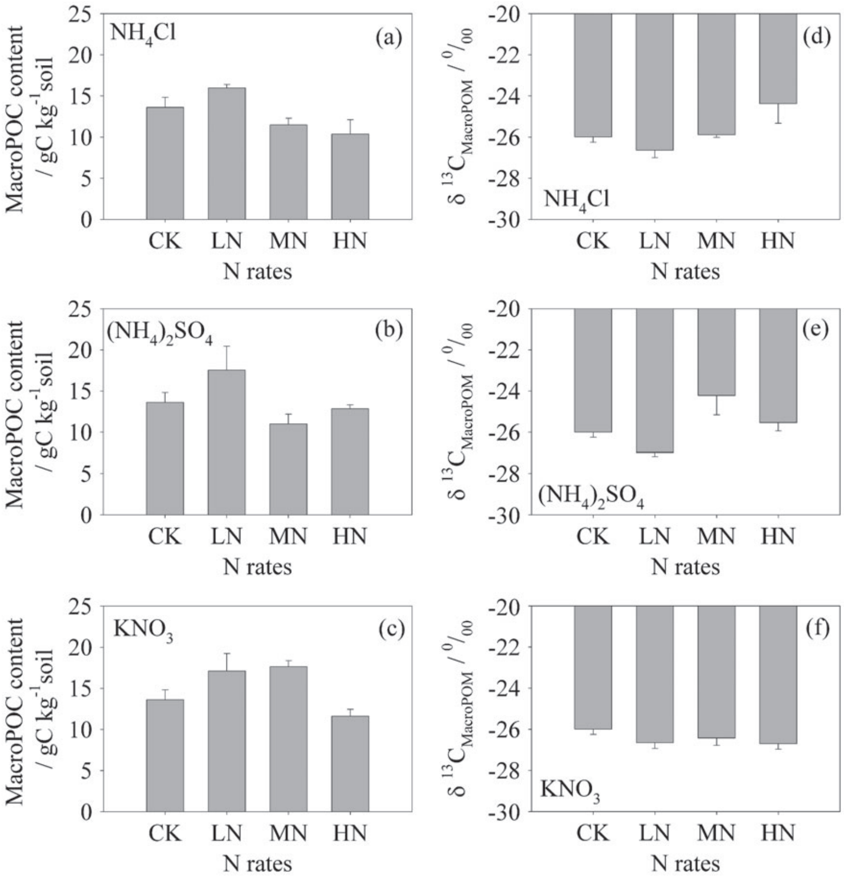

Figure 2 The SOC contents and average δ^{13}C values in $>250\,\mu$m MacroPOC fractions in the 0–10-cm layer under different N rates and N forms (a–f). Soil samples were collected from the alpine meadow on the Qinghai-Tibetan Plateau in 2011. Values are means of three replicates ± standard error.

the small N rate significantly depleted the average δ^{13}C values of the MacroPOC fraction, whereas medium and large N rates enriched the average δ^{13}C values of MacroPOC and MAOC fractions (Figure 6b, Table S2). However, N forms did not significantly change SOC stocks and the average δ^{13}C values of the whole soil, or the three particle-size fractions (Figure 6c,d, Table S2).

Discussion

Nitrogen addition effects on SOC fractions

Soil POC is a relatively unprotected C pool and sensitive to anthropogenic disturbances (Cambardella & Elliott, 1992). In our study, the amount of the different SOC fractions at 0–10-cm depth underwent significant changes after 5 years of ammonium-N fertilizer addition (Table 1 and Figure 1). The sensitivity of the alpine meadow ecosystem to N addition can be attributed to several factors. First, the meadow on the Qinghai-Tibetan Plateau is a fragile ecosystem with a shallow soil. Low temperature and small water availability leads to slow vegetation growth, and the surface accumulation of SOM makes the soil sensitive to natural and anthropogenic disturbances (Wang *et al.*, 2002). Second, POC is the most abundant SOC fraction, greater than MAOC, which is different from other grassland soils. For example, the proportion of POC to SOC in other alpine grassland soils is generally less than 40% (Gill, 2007), but more than 67% of SOC is POC in our study site. Our results align with the observation in the central Swiss Alps where Budge *et al.* (2011) found that alpine grassland soils contain a large proportion of labile C (about 58%).

For the N forms, we found that the addition of ammonium-N fertilizers had a greater effect on the SOC contents in the bulk soils and the three particle-size fractions than the nitrate-N fertilizers (Figures 1–4). Similarly, Currey *et al.* (2010) reported that NH_4^+ amendments had a stronger promotional effect on the potential use of labile C than NO_3^- amendment in an ombrotrophic peatland, with an increase in mineralization of most C substrates. Fang *et al.* (2012) also found that the stimulation effect on CO_2 effluxes was greater from NH_4^+ than from NO_3^- addition. The contrasting effects of NO_3^--N and NH_4^+-N accumulation on rates of C turnover are attributed to their opposite ion charges (Currey *et al.*, 2010), different mobility and soil ion exchange capacity (Killham, 1994), as well as the preferred use of microorganisms and plants (Xu *et al.*, 2011). Using a global meta-analysis of 15N tracer experiments, Kuzyakov & Xu (2013) recently suggested that microorganisms and plants had a greater capacity for NH_4^+-N uptake than for NO_3^--N uptake across the whole N concentration range, particularly at small concentrations in soils. In the alpine meadow ecosystem, plants prefer to absorb soil NH_4^+-N (Xu *et al.*, 2011), and applied ammonium-N fertilizer can increase plant biomass and ecosystem C sequestration (Fang *et al.*, 2012).

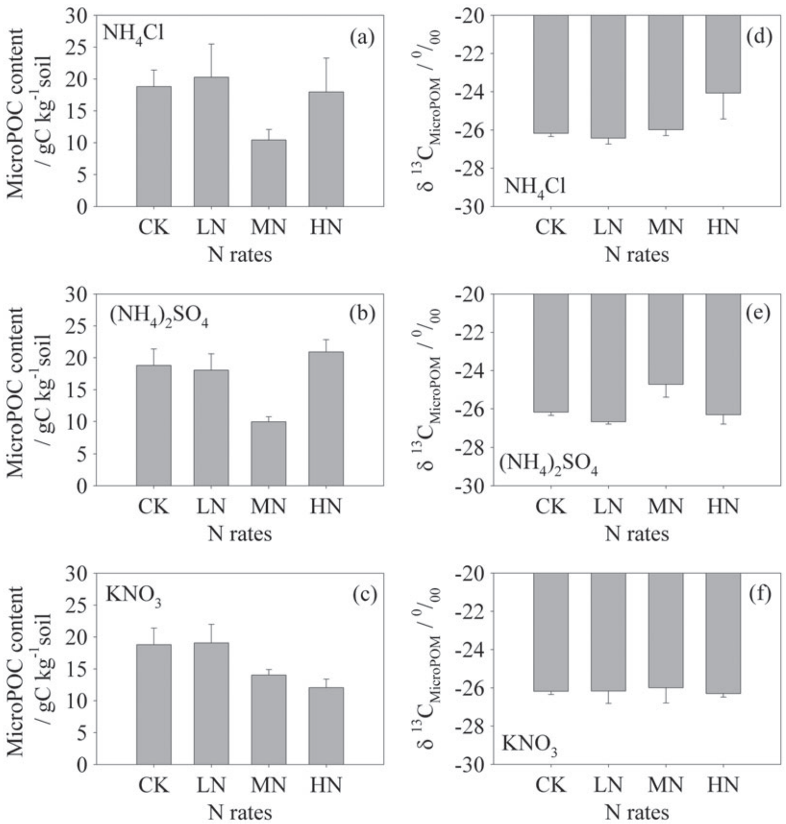

Figure 3 The SOC contents and average $\delta^{13}C$ values in 53–250 µm MicroPOC fractions in the 0–10-cm layer under different N rates and N forms (a–f). Soil samples were collected from the alpine meadow on the Qinghai-Tibetan Plateau in 2011. Values are means of three replicates ± standard error.

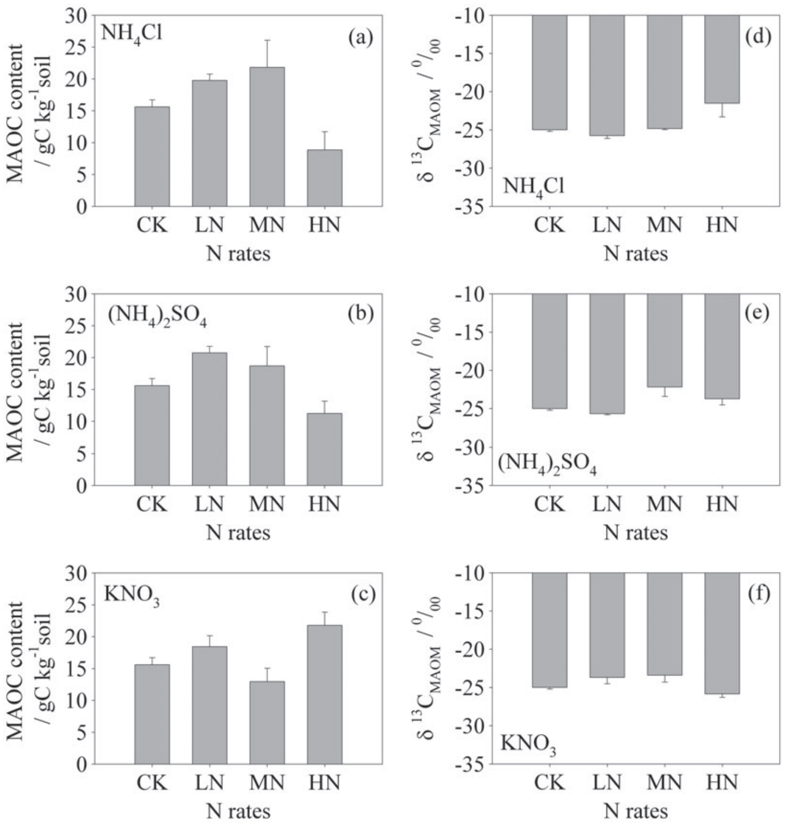

Figure 4 The SOC contents and average $\delta^{13}C$ values in < 53 µm MAOC fractions in the 0–10-cm layer under different N rates and N forms (a–f). Soil samples were collected from the alpine meadow on the Qinghai-Tibetan Plateau in 2011. Values are means of three replicates ± standard error.

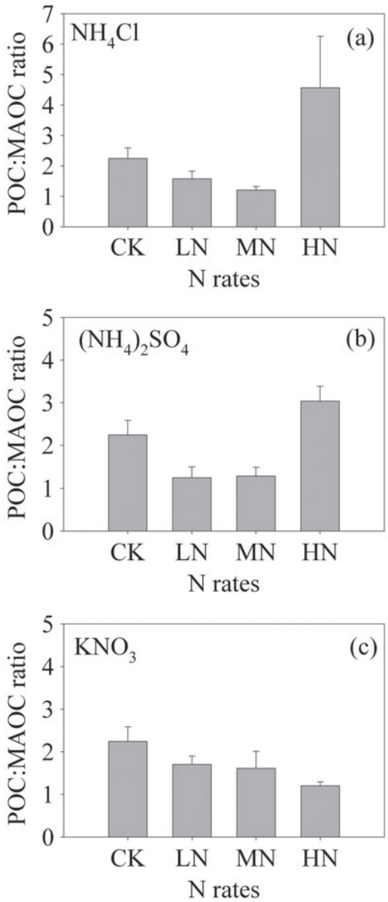

Figure 5 The POC/MAOC ratios in bulk soils in the 0–10-cm layer under different N rates and N forms (a–c). Soil samples were collected from the alpine meadow on the Qinghai-Tibetan Plateau in 2011. Values are means of three replicates ± standard error.

The increase in plant litter-C caused by N addition tended to stimulate soil microbial activities and to increase the utilization of C substrates through the so-called 'priming effect' (Kuzyakov & Xu, 2013). In addition, N forms significantly changed soil MAOC contents (Table 1), thus suggesting that soil MAOC was also sensitive to N addition regardless of NH_4^+ and NO_3^-. Our results agree with some studies that suggest passive SOC fractions have the same sensitivity to soil microbial decomposition (Fang *et al.*, 2005).

For the N rates, our data showed that a small N rate tended to increase total SOC stocks and POC contents (53–2000 μm), whereas medium and large rates had an opposite effect (Figure 6). Nitrogen addition may alter the content of SOC and its fractions by influencing the net primary production (NPP) and the decomposition of SOM (Piñeiro *et al.*, 2009). A small N rate tended to increase above-ground biomass and inhibited soil CO_2 efflux, but medium and large rates significantly promoted soil CO_2 release (Fang *et al.*, 2012). This explains partially the response patterns of SOC dynamics to different rates of applied N. In the N-limited alpine meadow ecosystem, it seems that plants and microorganisms can adapt to increased N availability up to a certain N input rate, and that soil functions will shift from C sequestration to C loss over the threshold of N retention. The critical level of N input appears to be between 10 and 20 kg N ha^{-1} year^{-1} including the ambient level of atmospheric N deposition (about 10 kg N ha^{-1} year^{-1}) at the Haibei station (Lü & Tian, 2007). This value falls within the critical loads of N deposition in global grasslands (5–30 kg N ha^{-1} year^{-1}; Bobbink & Hettelingh, 2010). Barrett & Burke (2002) estimated the threshold for N retention of pulse N addition in a long-term experiment in the surface soils of semi-arid grassland systems to be < 200 kg N ha^{-1}. Therefore, we can assume that the exogenous N input at the level of 20 kg N ha^{-1} year^{-1} will not adversely influence C sequestration in the alpine meadow soils on the Qinghai–Tibetan Plateau over 10 years. In addition, our results also show that the change of SOC stock caused by various rates of applied N was mainly attributed to the MacroPOC and MicroPOC (Figure 6). Moreover, the responses of soil MacroPOC to N application were more sensitive than those of soil MicroPOC (Figures 2, 3, Table 1). These results indicate that the new C resulting from the small N rates accumulated mainly in the MacroPOM fraction. Similarly, Cusack *et al.* (2011) reported that the increase in SOC content caused by simulated N deposition mainly occurred in the coarse-sized and small-density fractions.

Isotopic evidence on SOC dynamics caused by N addition

The ^{13}C natural abundance technique provides a way to characterize the dynamics of SOC with different turnover times (Balesdent *et al.*, 1996; Fang *et al.*, 2009). Generally, coarse-sized and small-density fractions of SOM originating from newly-formed plant residues are more ^{13}C-depleted, whereas SOM in fine-sized and large-density fractions is derived from more decomposed plant materials and is more ^{13}C enriched (Cadisch *et al.*, 1996). Our data show that the $\delta^{13}C$ values of MAOC were significantly larger than those of MacroPOC and MicroPOC (Figures 2–4). In addition, a large rate of NH_4Cl and a medium rate of $(NH_4)_2SO_4$ addition tended to enrich the $\delta^{13}C$ values of the total SOC and the three particle-sized fractions (Figures 1–4), which could be related to the increasing emission of ^{13}C-depleted CO_2 and the changes in C_3-C_4 plant communities caused by applied N. It has been found that the respired CO_2 is relatively ^{13}C depleted with respect to bulk soil and residual products of microbial decomposition (Wynn *et al.*, 2006). In one of our previous studies (Fang *et al.,* 2012) we showed that a small N-rate decreased soil CO_2 effluxes, while medium and large N rates promoted soil CO_2 emissions; this effect was more significant with NH_4^+-N than with NO_3^--N fertilizer. Further, the changes in plant communities caused by applied N could to some extent contribute to the changes of soil $\delta^{13}C$ values (Song *et al.*, 2012). However, the listed dominant plant species belong to C_3 plants; Yi *et al.* (2003) noted that no C_4 plants were present in the alpine meadow on the Qinghai-Tibetan Plateau. Therefore, we ruled out this possibility, and concluded that the ^{13}C-depleted CO_2 loss was probably the major reason for the ^{13}C enrichment in these three C fractions and total SOC.

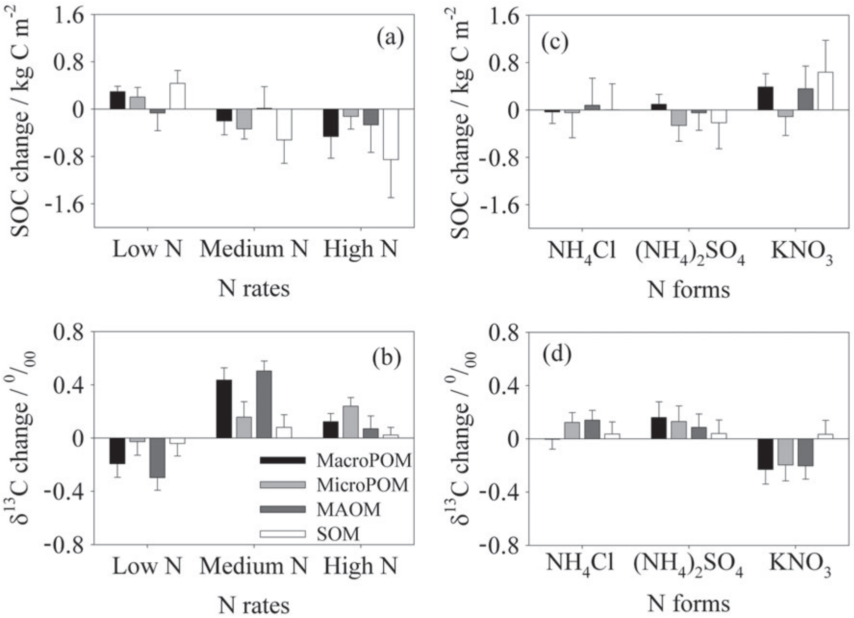

Figure 6 The net change of C stock and average $\delta^{13}C$ values in bulk soil and three particle-size fractions at the 30-cm depth after 5 years of applied N (a–d). Values are means ± standard error (n = 9).

Effect of applied N on SOM stabilization

In addition to altering SOM quantity, applied N in the alpine meadow ecosystem may change the composition of SOM because POC is a potential source of readily available C for decomposers and is more mineralizable than heavy SOM fractions (Whalen *et al.*, 2000). Thus, changes in the POC:SOC ratio resulting from applied N may potentially affect the stability of SOM (Martinsen *et al.*, 2011). When there is a large POC:MAOC ratio, the SOM will be less stable (Cheng *et al.*, 2010). Our results show that a large rate of ammonium-N fertilizer application significantly increased the POC:MAOC ratios of SOM, and that it was attributed mainly to the decrease in soil MAOC content (Figures 4, 5). However, applied nitrate-N fertilizer did not significantly change SOM stability despite a decreasing trend with N rate (Figure 5c), which can be explained by the small rate and short period of applied N. In contrast, some long-term applied N studies that used spectroscopic methods such as UV and fluorescence spectroscopy to characterize dissolved organic matter (DOM) properties, indicated that mineral N inputs increased the aromaticity of DOM and the complexity and thus the degree of condensation of the molecules (Hagedorn *et al.*, 2002). Using ^{13}C nuclear magnetic resonance (NMR) spectroscopy, Michel *et al.* (2006) also found that the composition of DOM was similar to that of its parent SOM, and applied N depleted polycondensated and aromatic compounds in DOM leached from forest floor samples, thus indicating that applied N may result in deposition of aromatic compounds in soil. Overall, experimental N deposition will eventually decrease the extent of litter and SOM decay, with an increase in alkyl C content and a decrease in O-alkyl C content (Baldock, 2007). Therefore, the accumulation of NH_4^+-N and NO_3^--N affects the stability of SOM adversely, but this will depend upon the duration and type of N fertilizer application.

Conclusions

This study characterized changes in the contents and $\delta^{13}C$ signatures of SOM and its fractions after 5 years of applied N in an alpine meadow on the Qinghai-Tibetan Plateau. Five years of applied N changed significantly the SOC contents in bulk soils and different particle-size fractions at the surface 10 cm depending on the rates and forms of N application. Ammonium-N fertilizer addition had more significant effects on the SOC contents and $\delta^{13}C$ values in the whole soil and different particle-size fractions than nitrate-N fertilizer addition. The critical level of N input for the alteration of SOC quantity was approximately 20 kg N ha^{-1} $year^{-1}$ while taking the ambient N deposition rate of 10 kg N ha^{-1} $year^{-1}$ into account. Therefore, exogenous N input at a rate less than this critical load is beneficial for SOM accumulation in the alpine meadow on the Qinghai-Tibetan Plateau over the short term. These results can provide a theoretical basis for the C and N management of alpine meadows in the future; however, further study is needed to explore the microbial mechanisms involved in the changes and stability of SOC caused by applied N. Moreover, a more complete study of the greenhouse gas balance and other potential impacts of N deposition on, for example, water quality or biodiversity is also necessary.

Supporting Information

The following supporting information is available in the online version of this article:

Table Sl. Summary results from one-way ANOVA on SOC contents and $\delta^{13}C$ values in bulk soils and various particle-size fractions in the 0–10-cm layer.

Table S2. Summary results from one-way ANOVA on changes of SOC stocks and average $\delta^{13}C$ values in bulk soil and three particle-size fractions at the 30-cm depth.

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