SOILS, SEC 1 • SOIL ORGANIC MATTER DYNAMICS AND NUTRIENT CYCLING • RESEARCH ARTICLE

Warming and grazing affect soil labile carbon and nitrogen pools differently in an alpine meadow of the Qinghai–Tibet Plateau in China

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Abstract

Purpose Small but highly bioactive labile carbon (C) and nitrogen (N) pools are of great importance in controlling terrestrial C and N fluxes, whilst long-term C and N storage is determined by less labile but relatively large sizes of C and N pools. Little information is available about the effects of global warming and grazing on different forms of C and N pools in the Qinghai–Tibet Plateau of China. The aim of this study was to investigate the effects of warming and grazing on the sizes of different soil labile C and N pools and N transformation in this region.

Materials and methods A free-air temperature enhancement system in a controlled warming–grazing experiment had been implemented since May 2006. Infrared heaters were used to manipulate temperature, and a moderate grazing intensity was simulated by Tibetan sheep. After 3 years' warming, soil samples were taken from the four treatment plots: no warming with no grazing; no warming with grazing; warming with no grazing; and warming with grazing. Concentrations of inorganic N in the 40–cm soil profiles were measured by a flow injection analyser. Microbial biomass C (MBC) and microbial biomass N (MBN) were measured by the fumigation–extraction method, and soluble organic C (SOC) and soluble organic N (SON) were determined by high-temperature catalytic oxidation. Total N (TN), C isotope composition (δ^{13}C) and N isotope

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composition (δ^{15}N) were determined using an isotope ratio mass spectrometer. Net N transformation under low temperature was studied in a laboratory incubation experiment.

Results and discussion Warming and grazing treatments affected soil C and N pools differently, and these effects varied with soil depth. Warming significantly increased TN, MBC, MBN, and SON and decreased δ^{13}C at the 10–20 and 20–30 cm soil depths, whilst grazing generally decreased SON at the 10–20 and 20–30 cm, and MBC at 20–30 cm. At the 0–10 cm depth, neither warming nor grazing alone affects these soil parameters significantly, indicating that there could be considerable perturbation on the soil surface. However, grazing alone increased NO_3^-–N, total inorganic N, SOC and δ^{15}N at the 0–10 cm depth. Incubated at 4°C, warming (particularly with grazing) led to net immobilization of N, but no-warming treatments led to net N mineralization, whilst nitrification was strong across all these treatments. Correlations between MBC and SOC, and TN and MBN or SON were positive. However, SON was less well correlated with TN and MBN compared with the highly positive correlations between SOC and MBC.

Conclusions It is clearly demonstrated that warming and grazing affected labile C and N pools significantly, but differently after 3 years' treatments: Warming tended to enlarge labile C and N pools through increased litter inputs, whilst grazing tended to increase inorganic N pools, decrease SON and accelerate N cycling. Grazing might modify the mode that warming affected soil C and N pools through its strong impacts on microbial processes and N cycling. These results suggested that interactive effects of warming and grazing on C and N pools might have significant implications for the long-term C and N storage and productivity of alpine meadow ecosystem in the Qinghai–Tibet Plateau of China.

Keywords Alpine meadow · Carbon cycling · Grazing · Nitrogen cycling · Warming

1 Introduction

Labile carbon (C) and nitrogen (N) pools, including inorganic N, microbial biomass C and N (MBC and MBN), and soluble organic C and N (SOC and SON), all play vital roles in soil C and N cycling, structure and functioning of ecosystems through their impacts on turnover and supply of nutrients to vegetation, and can be vulnerable to climate change and disturbance (Pastor and Post 1986; Zak et al. 1993; Hu et al. 1997; Pu et al. 2001; Chen et al. 2002; Blumfield and Xu 2003; Burton et al. 2007). Terrestrial C and N budgets are largely controlled by

the small but highly bioactive labile pools of these elements in soils (Mathers et al. 2003; Chen and Xu 2005; Belay-Tedla et al. 2009; Xu et al. 2009). Being a direct reservoir of readily available substrates and nutrient, labile C and N pools are especially important and may exert considerable control on the productivity, community structure and functioning of ecosystems through their impacts on turnover and supply of nutrients to vegetation (Pastor and Post 1986; Chen et al. 2003; Xu and Chen 2006; Burton et al. 2010). Global warming could stimulate C sequestrations in soil through enhanced primary production, but there is still uncertainty due to the potential increase of soil respiration (Rustad et al. 2001). Relative small changes in the sizes and distribution of soil C and N pools may therefore induce substantial effects on atmospheric greenhouse gas concentrations and on global C and N cycling at large (Belay-Tedla et al. 2009; Xu and Chen 2006). Such alterations in labile and less labile C and N pools may not only lead to new dynamic processes in the short term but can also influence the long-term terrestrial C and N storage and consequently feedback to the atmosphere (Xu et al. 2008, 2009; Burton et al. 2010; Jiang et al. 2010; Xing et al. 2010). Studies have also demonstrated that labile C and N pools in some situations could be sensitive to alterations in soil moisture, temperature and plant community structure resulting from climate change (Zak et al. 1993; Xu et al. 2009). Hence, measurements of the labile C and N pools may provide insights into the early indications of impacts of climate change on soil C and N dynamics and the consequent ecosystem functioning (Xu et al. 2008, 2009; Ge et al. 2010).

Inconsistent results on the responses of soil C and N dynamics to climate change have been observed so far (Rustad et al. 2001; Xu et al. 2009; Liu et al. 2010). Warming enhanced plant productivity (Chapin et al. 1995; Hartley et al. 1999; Jonasson et al. 1999) and litter mass losses (Luo et al. 2010), and thus a higher flux of carbon dioxide into the atmosphere (Meentemeyer 1978; Berg et al. 1993; Shaw and Harte 2001; Liski et al. 2003). These effects are reported most likely to take place in cold biomes (high-latitude and high-altitude sites) because the greatest warming is predicted to occur there, and decomposition in these regions is strongly temperature-limited (Hobbie and Chapin 1998; Robinson 2002; Aerts 2006). Grazing could affect the nutrient cycling mainly through providing plants with urine and faecal material and also by influencing litter mass losses (Hobbs et al. 1991; Bardgett et al. 2001; Olofsson et al. 2001), and its role depended on plant and litter quality. However, there is a scarcity of information about labile C and N pools and their coupling relations under future warming and grazing conditions in the Qinghai–Tibet Plateau of China and even in the world. Effects of warming and grazing on soil labile C and N pools

may occur in four ways: (1) by altering rates of litter mass loss directly at short timescales through changes in soil temperature and moisture (Kalbitz et al. 2000; Aerts 2006; Luo et al. 2010); (2) by decreasing litter mass with the increase in grazing intensity at short timescales (Shariff et al. 1994; Olofsson et al. 2001); (3) by changing plant litter quantity and quality indirectly at longer timescales (Aerts 2006); and (4) by changing indirectly the species composition and structure of the decomposer and detritivore communities in the long term (Kalbitz et al. 2000; Aerts 2006).

Global surface temperature is predicted to increase by 1.1°C to 6.4°C by the end of this century (IPCC 2007). The Qinghai–Tibet alpine meadow, unique among all ecosystems, is a sensitive region to global climate change (Thompson et al. 1993, 2000; Wang and French 1994). Moreover, a much greater than average increase in surface temperature was predicted to occur in this region in the future (Giorgi et al. 2001). Grazing is the main land use mode; there are about 13.3 million domestic yaks and 50 million sheep on the Qinghai–Tibet plateau, and grazing pressure will increase due to increasing population (Gerald et al. 2003; Yao et al. 2006). A number of simulated experiments have been carried out to study the impacts of global warming and grazing on terrestrial ecosystems all over the world. However, this is the first time that infrared heaters were used in the Qinghai–Tibet Plateau to simulate relevant ecosystem responses.

We experimentally manipulated temperature by actively warming plots using infrared heaters in a free-air temperature enhancement (FATE) system since May 2006, as described by Kimball et al. (2008), and applied a moderate grazing treatment to an alpine meadow on the Qinghai–Tibet Plateau. Interaction between warming and grazing on different soil C and N pools was studied at four soil depths: 0–10, 10–20, 20–30 and 30–40 cm. We hypothesized that warming would increase the sizes of labile C pools by increasing plant biomass (Luo et al. 2009; Hu et al. 2010), whilst grazing would alter the sizes of N pools and N transformation and modify the responses of soil C and N pools to warming. These effects could be different among the four soil depths.

The objectives of this study were to investigate through a controlled warming–grazing experiment: (1) the effects of warming and grazing on soil temperature and moisture, total N, C isotope composition ($\delta^{13}C$) and N isotope composition ($\delta^{15}N$) in the top 40 cm of the soil profile; (2) the effects of warming and grazing on soil inorganic N, SOC and SON, MBC and MBN, and N transformation under low temperature (4°C); and (3) the interactions between warming and grazing on these different forms of C and N pools and the relationships among these C and N pools.

2 Materials and methods

2.1 Experimental site

The experimental site and design were described by Kimball et al. (2008) and Luo et al. (2009, 2010). It is located at the Haibei Alpine Meadow Ecosystem Research Station (HBAMERS), a facility run by the Northwest Institute of Plateau Biology, Chinese Academy of Sciences. The station is situated at latitude 37°37′ N, longitude 101° 12′ E, and the mean elevation of the valley bottom is 3,200 m. Lying in a large valley surrounded by the Qilian Mountains on the northeast of the Qinghai–Tibetan Plateau, the station experiences a typical plateau continental climate which is dominated by the southeast monsoon in summer, from May to September, and high pressure from Siberia in winter. Summers are short and cool and winters are long and severely cold. The mean annual temperature is −2°C, whilst the mean annual precipitation is 500 mm, over 80% of which falls during the summer monsoon season.

The infrared heating system, herein called a free-air temperature enhancement (FATE), has been set up since May 2006. The set point differences between the heated and corresponding reference plots were 1.2°C during daytime and 1.7°C at night in summer, which fell within the limits of the predicted temperature increases for this century (1.5–5°C; Houghton et al. 2001). The soil temperature at depths of 5, 10 and 20 cm were measured automatically using type K thermocouples (Campbell Scientific, Logan, Utah, US.A) which were connected to a CR1000 data logger. Meanwhile, the soil temperatures at the 0– and 40–cm depths were manually monitored using mercury-in-glass thermometers. Warming significantly increased soil temperatures for the 0–40 cm soil depths in 2006, 2007 and 2008. Mean seasonal soil temperature increases in the warmed plots above the reference plots were 0.8–1.3°C, 1.4–1.5°C, 1.2–1.4°C, 1.1–1.2°C and 0.5–0.7°C at the 0-, 5-, 10-, 20 and at 40-cm soil depths in 2006 and 2007, respectively. More data can be found in our published results (Luo et al. 2009, 2010; Hu et al. 2010).

A two factorial design (warming and grazing) was used with four replicates for each of four treatments, i.e. no warming with no grazing (NWNG); no warming with grazing (NWG); warming with no grazing (WNG); and warming with grazing (WG). In total, 16 circular plots of 3–m diameter were used in a randomized block design in the field.

Initially, one adult Tibetan domestic sheep (*Ovis aries*) was fenced in the grazing plots on the morning of 15 August 2006 for approximately 2 h. The canopy height was 8–9 and 4–5 cm before and after grazing, respectively. Two sheep were fenced for approximately 1 h in the grazing plots on the mornings of 12 July, 3 August and 12 September 2007, and 8 July and 20 August in 2008. The

canopy height of the vegetation was measured at 50 points within the plots before and after grazing, and the sheep were removed from the grazing plots when the canopy height was reduced to approximately half of the initial height, which generally corresponded to a moderate stocking rate in the region. All experimental sheep were fenced into three additional 5×5-m fenced plots for a day before beginning the grazing experiment to help them adapt to small plots.

2.2 Soil sampling

Soil samples from each plot were collected on 2 August 2009 using a drill. Five soil cores were randomly collected within each plot and bulked as a single sample. As the impact of infrared radiation on soil temperature reached the depth of 40 cm (Luo et al. 2010), soil samples from four soil depths—0–10, 10–20, 20–30 and 30–40 cm—were taken. All soil samples were sent to the laboratory and sieved through a 2-mm screen and stored in a refrigerator at 4°C prior to analyses. Soil subsamples were extracted within 24 h for NH_4^+–N and NO_3^-–N analysis.

2.3 Soil analysis

Soil pH was determined in 1:5 (*v/v*) soil/water extracts using a combination glass electrode, and soil gravimetric moisture was determined by drying at 105°C for 24 h. Soil NH_4^+–N and NO_3^-–N were determined in 2 M KCl extracts by LACHAT Quickchem Automated Ion Analyzer (Quick-Chem Method 10-107-06-04-D for NH_4^+–N and Quick-Chem Method 12-107-04-1-B for NO_3^-–N). In addition, a low-temperature incubation experiment was carried out in the laboratory as the 0–10 cm soil samples were incubated for 3 months at 4°C, which was close to in situ mean annual temperature of this region. N transformation during the 3 months was analysed by calculating the difference of inorganic N concentrations before and after this period. Net ammonification, net nitrification and net N mineralization were calculated as the differences of soil NH_4^+–N, NO_3^-–N and total inorganic N before and after this period.

Soil total N (TN), $\delta^{13}C$ and $\delta^{15}N$ were determined using an isotope ratio mass spectrometer with a Eurovector Elemental Analyzer (Isoprime-EuroEA 3000, Milan, Italy).

Soil MBC and MBN were measured using the fumigation–extraction method described by Vance et al. (1987) and Brookes et al. (1985). In brief, fumigated and non-fumigated soils (4-g dry weight equivalent) were extracted with 20 ml of 0.5 M K_2SO_4 (soil/extractant ratio 1:5). The fumigation lasted for 16 h. Samples were shaken for 1 h and filtered through a Whatman 42 filter paper. Soluble organic C and total soluble N (TSN) in the fumigated and non-fumigated samples were determined using a SHI-

MADZU TOC$_{-VCPH/CPN}$ Analyzer. NH_4^+–N and NO_3^-–N concentrations in 0.5 M K_2SO_4 extracts were determined by LACHAT Quickchem Automated Ion Analyzer, whilst SON was calculated as the difference between TSN and soil inorganic N (SIN). MBC and MBN were calculated using a conversion factor for C (E_c) of 2.64 (Vance et al. 1987) and for N (E_n) of 2.22 (Brookes et al. 1985).

2.4 Statistical analysis

Statistical significances of the effects of warming and grazing on soil moisture at different soil depths, inorganic N concentrations, TN, $\delta^{13}C$, $\delta^{15}N$, MBC, MBN, SOC and SON were determined separately by analysis of variance (ANOVA) using Statistix for Windows, version 8.0 (Analytical Software, Tallahassee, FL, USA), with warming and grazing as the main factors. Least significance difference was used to separate the means when differences were significant. Significance was assumed at the $P=0.10$ level as there might be unavoidable disturbance in the field. Simple correlations between all these variables were performed.

3 Results

3.1 Soil temperature, moisture, TN and C and N isotope compositions

Warming significantly increased soil temperature at the 0–40 cm depth during the growing season of 2009 (Fig.1a). No interaction between warming and grazing was found on soil temperature, although NWG also increased it by decreasing vegetation canopy height, probably through increased solar radiation. WG caused the largest increase in soil temperature compared with other treatments.

Warming significantly decreased soil moisture at all depths ($F=59.59$, $P<0.01$; Table 1 and Fig. 1b). No direct influence of grazing and interaction between warming and grazing on soil moisture was found. However, the interactions between warming and depth; grazing and depth; and warming, grazing and depth on soil moisture were all significant ($P<0.001$, $P<0.10$ and $P<0.05$, respectively; see Table 1).

Generally, the effect of warming on TN varied with soil depths (Table 2). Warming significantly increased TN at the 10–20 cm ($F=21.9$, $P=0.018$) and 20–30 cm ($F=7.77$, $P=0.069$) depths (see Table 2 and Electronic supplementary material (ESM) Tables S1 and S3), but the interaction between warming and grazing was not significant. At 0–10 cm, however, the effects of warming and grazing were not significant; WNG decreased TN by 4.6% compared with NWNG, whilst WG increased them by 8.9% compared with NWG.
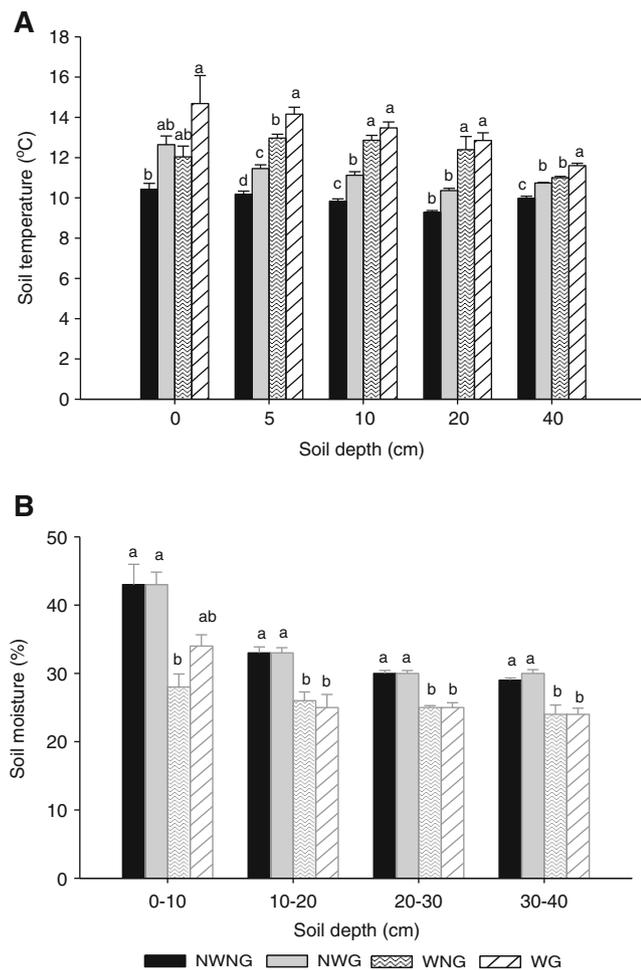
Fig. 1 Soil temperature (°C) of growing season (**a**) and gravimetric moisture content (%) (**b**) at different depths under different warming and grazing regimes in 2009. *NWNG* no warming with no grazing, *NWG* no warming with grazing, *WNG* warming with no grazing, *WG* warming with grazing. *Different letters* mean significant differences between treatments at $P<0.05$ for each soil depth. Mean ± SE is shown in the figure

There was a significant effect of warming in decreasing $\delta^{13}C$ at the 10–20 cm ($F=9.99$, $P=0.051$) and the 20–30 cm

($F=9.35$, $P=0.055$) depths (see Table 2 and ESM Table S3). Warming did not affect $\delta^{15}N$ significantly. However, at 0–10 cm, there was a significant increase of $\delta^{15}N$ in the grazing plot ($F=6.5$, $P=0.084$); NWG increased it by 18.6% compared with NWNG (see Table 2 and ESM Table S3). The interactions between warming and grazing on $\delta^{15}N$ were not significant at the 0–10 cm ($F=5.54$, $P=0.100$) and the 10–20 cm ($F=4.15$, $P=0.134$) depths (see ESM Table S3).

3.2 NH_4^+–N, NO_3^-–N and total inorganic N

Generally, soil inorganic N concentrations were more affected by grazing than by warming (Fig. 2a, b). No significant effect of warming on NH_4^+–N, NO_3^-–N and SIN concentrations was found, but soil depth had a significant effect. Concentration of NH_4^+–N among all soil depths ranged from 16.61 to 25.52 mg kg^{-1}, whilst that of NO_3^-–N ranged from 1.43 to 43.17 mg kg^{-1}.

Grazing posed a significant influence in increasing NO_3^-–N overall ($F=4.88$, $P=0.047$) at the 0–10 cm ($F=5.69$, $P=0.097$), the 10–20 cm ($F=6.99$, $P=0.078$) and the 20–30 cm ($F=7.40$, $P=0.073$) depths, respectively, as well as increasing SIN overall ($F=5.07$, $P=0.044$; see Fig. 2b, c). Specifically, at 0–10 cm, NWG increased NO_3^-–N by over twofold compared with NWNG, whilst WG drastically increased NO_3^-–N by over sixfold compared with WNG. As well, NWG increased SIN by 21.3% compared with NWNG, whilst WG increased it by 145% compared with WNG at 0–10 cm. Warming tended to decrease NH_4^+–N, NO_3^-–N and SIN, whilst grazing tended to increase them; however, WG caused the largest increases in NO_3^-–N and SIN (see Fig. 2b, c).

3.3 MBC, MBN, SOC and SON

The effects of warming on MBC and MBN varied with soil depth. Warming significantly increased MBC at the 10–20 cm ($F=11.1$, $P=0.045$) and the 20–30 cm ($F=8.22$,

Table 1 ANOVA for moisture content and inorganic N in the 40-cm soil profile under different warming and grazing regimes

Factors	Moisture (%)			NH_4^+–N (mg kg^{-1})			NO_3^-–N (mg kg^{-1})			Inorganic N (mg kg^{-1})		
	df	F	P	df	F	P	df	F	P	df	F	P
Warming	1	59.59	<0.001***	1	0.10	0.754	1	2.12	0.171	1	2.58	0.135
Grazing	1	0.67	0.427	1	0.02	0.898	1	4.88	0.047**	1	5.07	0.044**
Warming × grazing	1	0.24	0.630	1	1.94	0.189	1	1.91	0.192	1	3.41	0.089*
Depth	3	77.30	<0.001***	3	27.99	<0.001***	3	7.81	<0.001***	3	14.93	<0.001***
Warming × depth	3	9.21	<0.001***	3	1.22	0.316	3	2.41	0.083*	3	1.77	0.170
Grazing × depth	3	2.44	0.081*	3	0.05	0.983	3	5.05	0.005***	3	4.78	0.007***
Warming × grazing × depth	3	3.02	0.042**	3	1.00	0.404	3	1.81	0.163	3	2.28	0.096*

*$P<0.10$, **$P<0.05$, ***$P<0.01$

Table 2 Mean values for TN, C isotope composition (δ^{13}C), N isotope composition (δ^{15}N), MBC, MBN, SOC and SON in the 40-cm soil profile under different warming and grazing regimes (standard errors are shown in parentheses)

Treatments	TN (%)	δ^{13}C (‰)	δ^{15}N (‰)	MBC (mg kg^{-1})	MBN (mg kg^{-1})	SOC (mg kg^{-1})	SON (mg kg^{-1})
0–10 cm							
NWNG	0.693 (0.04)	−26.1 (0.28)	3.35 (0.15) B	2245 (322)	274 (60)	573 (74) b	26.2 (6.4)
NWG	0.690 (0.03)	−26.0 (0.19)	3.98 (0.13) A	2101 (133)	283 (57)	607 (42) ab	24.4 (3.1)
WNG	0.661 (0.03)	−25.9 (0.23)	3.50 (0.21) B	2211 (369)	249 (41)	560 (53) b	27.3 (3.6)
WG	0.755 (0.04)	−26.2 (0.14)	3.53 (0.21) AB	2407 (244)	316 (33)	718 (113) a	37.6 (11.3)
10–20 cm							
NWNG	0.449 (0.01) B	−23.9 (0.25) A	4.23 (0.16)	1098 (155) b	131 (18)	465 (61)	27.9 (2.8)
NWG	0.449 (0.01) B	−24.0 (0.14) AB	4.45 (0.04)	1049 (132) b	111 (12)	427 (22)	25.2 (2.3)
WNG	0.470 (0.02) AB	−24.2 (0.20) AB	4.29 (0.09)	1251 (116) ab	154 (16)	433 (20)	28.2 (3.4)
WG	0.499 (0.02) A	−24.7 (0.26) B	4.23 (0.15)	1373 (139) a	173 (35)	472 (37)	28.0 (4.3)
20–30 cm							
NWNG	0.375 (0.02) ab	−22.7 (0.30) AB	4.75 (0.09) ab	904 (77) AB	119 (16)	381 (31)	24.0 (1.7) A
NWG	0.347 (0.01) b	−22.4 (0.24) A	4.95 (0.23) a	783 (71) B	88.9 (15)	360 (29)	22.5 (1.6) AB
WNG	0.385 (0.02) a	−22.9 (0.32) AB	4.30 (0.30) b	1012 (137) A	117 (18)	369 (19)	23.7 (2.7) AB
WG	0.390 (0.02) a	−23.1 (0.12) B	4.63 (0.10) ab	950 (134) AB	115 (10)	384 (38)	21.6 (2.3) B
30–40 cm							
NWNG	0.301 (0.01)	−20.8 (0.36)	4.83 (0.17)	572 (45) B	73.6 (10)	320 (26)	16.8 (1.1)
NWG	0.299 (0.02)	−20.7 (0.39)	4.69 (0.17)	547 (49) B	67.4 (16)	312 (26)	19.4 (2.4)
WNG	0.323 (0.03)	−21.4 (0.56)	4.59 (0.26)	720 (94) A	81.1 (19)	319 (37)	19.3 (2.3)
WG	0.303 (0.02)	−20.8 (1.00)	4.72 (0.11)	613 (82) AB	68.8 (6)	310 (26)	20.0 (2.1)

Where values are followed by different uppercase or lowercase letters for each soil depth, this indicates that treatment means are significantly different from each other at $P<0.05$ or $P<0.10$, respectively

NWNG no warming with no grazing, *NWG* no warming with grazing, *WNG* warming with no grazing, *WG* warming with grazing

$P=0.024$) depths (ESM Table S4). Grazing decreased MBC significantly at 20–30 cm ($F=7.94$, $P=0.067$). No significant interactions between warming and grazing on MBC were found among any of the depths. Although the effect was not robust, warming increased MBN at the 10–20 cm ($F=5.88$, $P=0.094$) depth (see ESM Table S4). No significant effects of warming and grazing or interaction between them on MBN were found. However, at 0–10 cm, WG tended to increase MBN by 15.3%, 11.7% and 27.0%, respectively, compared with NWNG, NWG and WNG, respectively.

Warming and grazing affected SOC and SON differently and inconsistently among soil depths. At 0–10 cm, grazing significantly increased SOC ($F=6.56$, $P=0.083$), but there was no interaction between warming and grazing ($F=2.75$, $P=0.196$; see ESM Table S4). At 10–20 cm, the effects of warming ($F=12.11$, $P=0.040$) in increasing SON and the effects of grazing ($F=10.7$, $P=0.047$) in decreasing SON were both significant (see ESM Table S4). At the 20–30 cm depth, grazing also significantly decreased SON ($F=292$, $P=0.013$; see ESM Table S4).

3.4 N transformation under low temperature

After 3 months' incubation, the composition of inorganic N had changed greatly (see Fig. 2d) as low concentrations of NH_4^+–N and abundance in NO_3^-–N were observed, which was opposite to the state before incubation. The 4°C incubation experiment had shown that warming (particularly with grazing) led to net immobilization of N, but no-warming treatments led to net N mineralization (see Fig. 2d). However, WG caused the largest net N immobilization, and nitrification was strong across all these different treatments (see Fig. 2d).

3.5 Relationships among the soil C and N pools

There were positive correlations between TN and MBN ($R^2=0.76$, $P<0.001$) and between TN and SON ($R^2=0.24$, $P<0.001$; Fig. 3a). Positive correlation was also found between MBC and SOC ($R^2=0.53$, $P<0.001$) and between MBN and SON ($R^2=0.21$, $P<0.001$; see Fig. 3c, d). However, SON was less well correlated with TN and
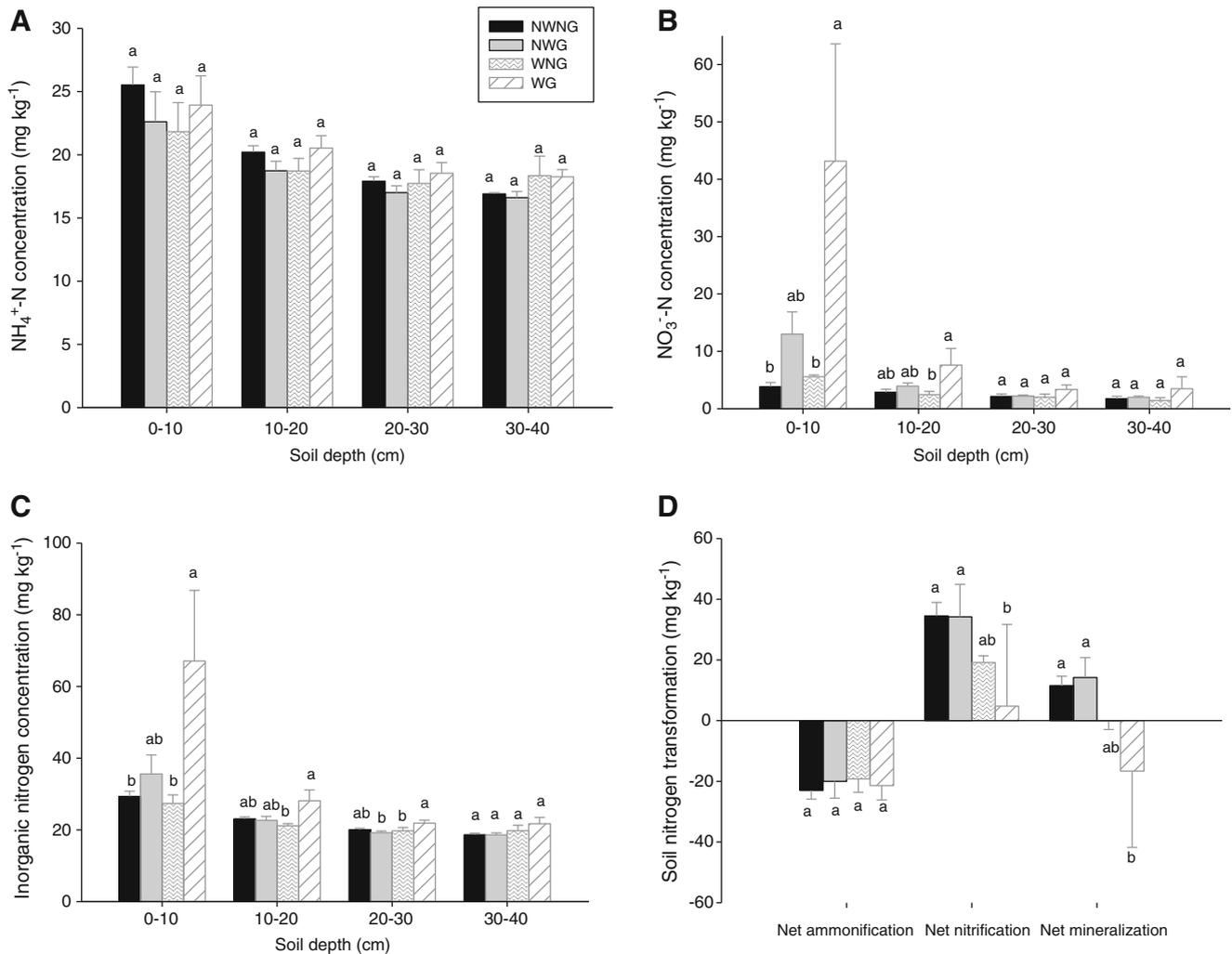
Fig. 2 NH_4^+–N (a), NO_3^-–N (b) and total inorganic N (c) concentrations at soil depths of 0–10, 10–20, 20–30 and 30–40 cm under different warming and grazing regimes, and net N transformations in the 0- to 10-cm soil under laboratory low-temperature conditions (4°C) for 3 months (d). *NWNG* no warming with no grazing, *NWG* no warming with grazing, *WNG* warming with no grazing, *WG* warming with grazing. *Different letters* mean significant differences between treatments at $P<0.05$ for each soil depth (a–c) and each N transformation process (d). Mean ± SE is shown in the figure

MBN compared with the highly positive correlations between SOC and MBC.

4 Discussion

4.1 Effects of warming on C and N pools

Some studies reported that labile C and N pools obtained through different hydrolysis methods as well as microbial biomass C and N pools were significantly increased by experimental warming and elevated CO_2 (Belay-Tedla et al. 2009; Zak et al. 1993) through accelerated decomposition rates or enhanced substrate inputs. According to our published results (Luo et al. 2009), WNG increased dissolved organic C (DOC) in 2006 and 2007. Here, we also found that warming increased various C and N pools including MBC, MBN and SON, as well as total N in the soil.

Microbial biomass C and N pools are vital components of ecosystem cycling and serve as a source (mineralization) or a sink (immobilization) of labile nutrients (Hu et al. 1997). Microbial biomass could respond rapidly to changes in soil moisture (Skopp et al. 1990) and soil temperature (Fang et al. 2005). A lag time for microbial biomass C and N in response to experimental warming had been reported in field experiments in subarctic soils (Ruess et al. 1999). However, in our study, warming increased both MBC and MBN at the 10–20 and 20–30 cm depths, suggesting that microbial immobilization of C and N was significantly enhanced by warming despite the control of decreased soil
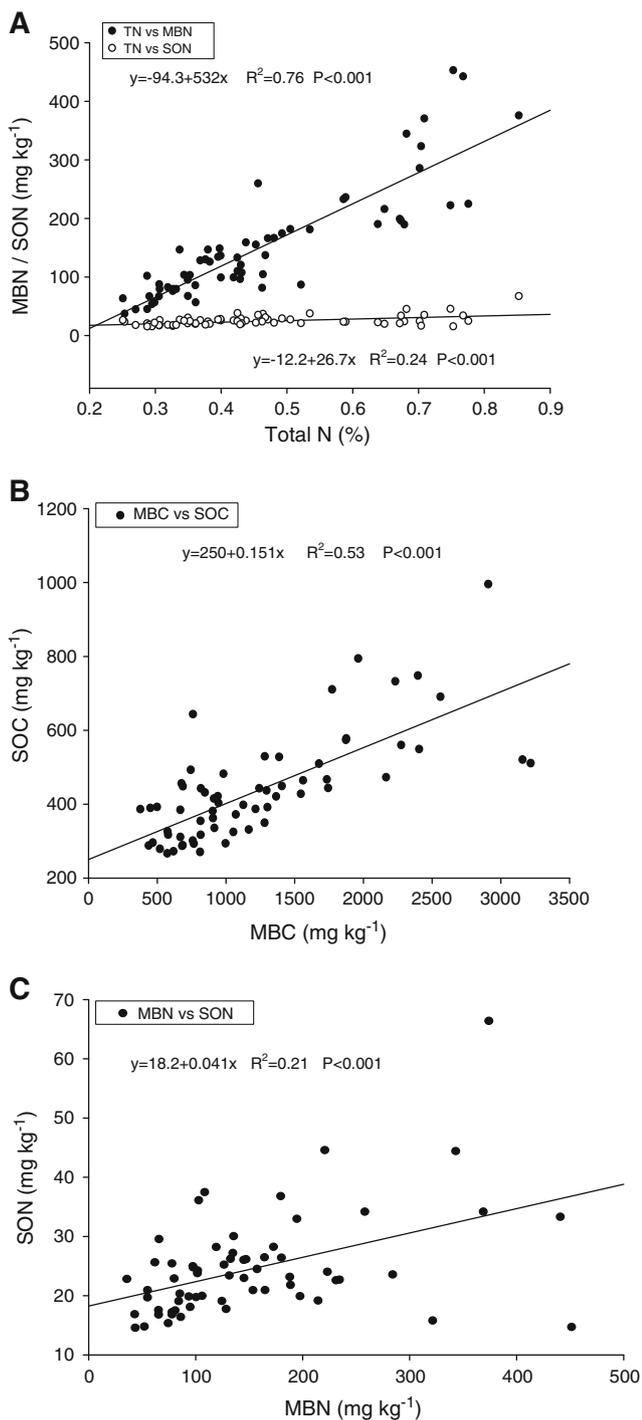
Fig. 3 Relationships between total nitrogen (*TN*) (%) and microbial biomass N (*MBN*) (mg kg^{-1}) and soluble organic N (*SON*, mg kg^{-1}) (**a**); MBC (mg kg^{-1}) and SOC (mg kg^{-1}) (**b**); and MBN (mg kg^{-1}) and SON (mg kg^{-1}) (**c**)

moisture on microorganisms. This was consistent with the results from the low-temperature incubation where warming led to net immobilization of N, whilst no warming led to net N mineralization during the incubation (see Fig. 2d). Microorganisms, especially nitrifiers, were largely stimulated

by warming as NH_4^+–N was continuously being consumed (see Fig. 2d), indicating a great demand by microbes in the warming plots to take up inorganic N. Besides the increase in the microbial biomass C and N pools, how warming affects the nitrifying groups and other microorganisms will be crucial for understanding the mechanisms of microbial-mediated C and N cycling. Further studies are required to investigate the effects of warming and grazing on nitrifying and denitrifying communities.

SOC and SON, often considered as direct substrates of N mineralization, were small but highly bioactive pools and were important in controlling short-term terrestrial C and N cycling (Chen and Xu 2008; Belay-Tedla et al. 2009). Several results had confirmed that some plants were able to directly utilize and generally prefer amino acid over inorganic N (Schimel and Chapin 1996; Chen and Xu 2008). It was also reported that in the alpine meadow of the Qinghai–Tibet Plateau, organic N uptake by plants was quantitatively significant under field conditions (Xu et al. 2004, 2006). Most of SOC and SON in soils were derived from root exudation, litter decomposition, transformation of organic matters and immobilization of inorganic C and N (Chen and Xu 2006, 2008). Therefore, the quantity and quality of organic and inorganic inputs and the associated microbially mediated processes, which were always controlled by temperature and moisture, were important in determining the sizes of SOC and SON. In our study, warming did not affect SOC, but significantly increased SON at the 10–20 cm depth, implying that both pools might not simply be controlled by a single factor but by a combination of temperature, moisture, substrate inputs and microbial processes. According to our previous findings (Luo et al. 2009), the direct contribution of temperature and moisture on DOC was small, whilst biotic factors (i.e. quality of standing dead and belowground biomass) were the main controls on DOC in the soil. Belay-Tedla et al. (2009) also suggested that the increases in labile and microbial biomass C and N pools under warming conditions largely resulted from increased above- and belowground biomass. On the other hand, higher soil temperature in warmed plots might stimulate the decomposition of the recalcitrant pools (Knorr et al. 2005), which could increase labile C and N levels, whilst decreased soil moisture under warming might restrict the decomposition of the labile and recalcitrant pools (Skopp et al. 1990). Therefore, the overall direct effects of temperature and moisture on labile C and N pools might be relatively small compared with the impact of changes in substrate inputs. However, the increased SON indicated a larger source for direct plant uptake or N mineralization under warming conditions.

The effect of warming in increasing total N, especially at the 10–20 and the 20–30 cm depths, might result from the shift in plant composition because

warming significantly increased the N-fixing plants in our study (data not shown). Despite the scarcity of published information about this effect, it was very likely that warming would cause change in plant community composition, which would lead to shifts in the composition of microorganisms through alterations in aboveground inputs and rhizospheric exudates that might favour different microorganisms. This 'plant–microbe' interaction could feedback in regulating C and N cycling in the long term (Xu and Chen 2006; Xu et al. 2009). In addition, at 0–10 cm, warming did not affect total N significantly, implying that near the soil surface, there could be large disturbance like grazing, causing considerable perturbations in top soil N contents. Warming tended to affect $\delta^{13}C$ by decreasing it significantly at the 10–20 and the 20–30 cm depths. Previous studies suggested that soil $\delta^{13}C$ was highly related with plant $\delta^{13}C$ through plant litter inputs, which had been demonstrated to be positively related to plant water use efficiency (WUE) and photosynthesis (Xu et al. 2000; Huang et al. 2008a, b; Tutua et al. 2008; Sun et al. 2010). Farquhar et al. (1989) indicated that the decrease in soil moisture under warming conditions could result in a greater WUE of plants, as well as enhanced C_4 photosynthesis, which could cause $\delta^{13}C$ to increase (Farquhar et al. 1989). However, within this region, C_3 species were believed to dominate. The decrease in $\delta^{13}C$ here under warming conditions was more likely to be caused by the increase in annual net primary production (data not shown), which was a direct result of enhanced photosynthesis. Therefore, discrimination against ^{13}C by the carboxylating enzyme in C_3 plants (Farquhar et al. 1982; Dawson et al. 2002) could be enhanced as a result of stimulated photosynthesis, leading to the decrease of $\delta^{13}C$ in plants and subsequently in soil. The decrease of $\delta^{13}C$ suggested that water content might not act as a limiting factor to plants here. The C isotope composition of plants could provide an opportunity to assess the long-term stability of plant communities and climate of a region (Nordt et al. 1994). Therefore, the signature of soil $\delta^{13}C$ under warming conditions in our study reflected a significant implication of warming on plants of this ecosystem.

4.2 Effects of grazing on C and N pools

It was well documented that grazing could enhance the decomposition and mineralization of SOM through its impact on quality and quantity of litter (Hobbs et al. 1991; Shariff et al. 1994; Bardgett et al. 2001; Olofsson et al. 2001), but inconsistent results had been reported on the effects of grazing on C and N pools. Some suggested that grazing could increase C and N pools (Reeder et al. 2004), whilst others showed opposite results (Stark et al. 2003; Golluscio et al. 2009), indicating that the effects of grazing on C and N

pools could vary with ecosystems. Here, we found in the alpine meadow ecosystem of the Qinghai–Tibet Plateau that grazing greatly increased inorganic N, the effect of which was intensified by warming. Grazing also increased SOC and $\delta^{15}N$ at 0–10 cm, but decreased SON at the 10–20 and 20–30 cm depths, and MBC at 20–30 cm.

The significant increase in $NO_3^- –N$ concentrations under NWG and WG compared with non-grazing treatments could be explained by the direct effect of sheep dung deposition which probably contains high concentrations of inorganic N and could rapidly increase nitrate concentration on the soil surface (Hobbs et al. 1991; Bardgett et al. 1997; Olofsson et al. 2001). Microbial activity could also be stimulated by saliva excreted by grazing animals. In addition, there could be a general positive feedback as herbivory could promote plant regrowth as well as energy and nutrient flow in grazed plots (Hamilton and Frank 2001). Although NWG did not affect the total inorganic N significantly, WG would increase it significantly ($P=0.089$), implying that the effects of grazing on inorganic N could be intensified by warming. The interaction between warming and grazing could possibly stimulate microbial activities and N mineralization strongly (Rustad et al. 2001; Melillo et al. 2002), resulting in a substantial feedback in inorganic N pools. The effects of grazing on inorganic N were more significant at the 0–10 cm depth, implying the direct impact of grazing on the highly dynamic and transient turnover of labile N near the soil surface.

Grazing increased SOC at 0–10 cm, but decreased SON at the 10–20 and 20–30 cm depths and MBC at 20–30 cm, which was different from the effects of warming which generally increased these labile organic C and N pools. The removal of aboveground biomass by grazing would directly decrease the litter mass input. However, the increase in SOC at the 0–10 cm depth was more likely to be caused by the changed quality of litter input. The decreases in SON and MBC could be explained by less litter inputs as well as the effects of grazing in enhancing mineralization (Shariff et al. 1994; Hamilton and Frank 2001). The regrowth of plants and the flow of nutrients promoted by grazing, as well as the enhanced microbial activity stimulated by enzymes contained in saliva and dung, would greatly promote plant uptake of SON and its microbial mineralization. These results suggest that unlike the effects of warming which tended to favour increases in labile C and N pools through increasing substrate input, grazing would affect them by influencing the quality of litter input and stimulating the nutrient and energy flows in the soil (Hamilton and Frank 2001). However, SON was less well correlated with TN and MBN compared with the highly positive correlations between SOC and MBC, indicating the strong influences of grazing on N cycling. Further investigations should be conducted into C and N cycling and

storage under future warming and grazing conditions in the alpine meadow of the Qinghai–Tibet Plateau.

Unlike warming, grazing did not affect $\delta^{13}C$ but increased $\delta^{15}N$ at the 0–10 cm depth, which might also imply that N cycling could be directly accelerated by grazing rather than warming. Soil $\delta^{15}N$ was presumed to be an index of N cycling as a higher cycling rate might yield greater N loss (Nadelhoffer and Fry 1994; Dawson et al. 2002). The change in soil $\delta^{15}N$ under grazing conditions could be attributed to both the N inputs in the forms of sheep excreta, as well as the shift in the compositions of the plants and microbes because of grazing (Frank and Evans, 1997). Soil could become enriched in ^{15}N when ^{15}N-depleted products (i.e. NH_3, NO_3^-, N_2O, N_2), resulting from fractionation occurring during soil N transformations, were lost from the soil system (Evans and Ehleringer 1993; Nadelhoffer and Fry 1994). Our previous study indicated that NWG and WG significantly increased the average annual N_2O flux (57.8% and 31.0%) compared with NWNG and WNG, respectively (Hu et al. 2010). Thus, considering there is now increasing grazing pressure in this region, N cycling could be accelerated in the long term, resulting in higher N availability as well as larger N_2O emissions.

5 Conclusions

After 3 years' treatment, warming and grazing affected labile C and N pools significantly and differently. Warming significantly increased TN, MBC, MBN, and SON and decreased $\delta^{13}C$ at the 10–20 and 20–30 cm soil depths, whilst grazing generally decreased SON at 10–20 and 20–30 cm and MBC at the 20–30 cm depth. Grazing alone increased NO_3^-–N, total inorganic N, SOC and $\delta^{15}N$ at the 0–10 cm depth. Strong interactive effects of warming and grazing on soil C and N pools found in this study might have significant implications for the long-term C and N storage and productivity of the alpine meadow ecosystem in the Qinghai–Tibet Plateau of China. In future works, how seasonal patterns of labile C and N, as well as microbial communities that mediate the key processes in soil C and N cycling, respond to warming and grazing will be crucial to increase our knowledge on the mechanisms and long-term effects of warming and grazing on this plateau.

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