Nine years of warming and nitrogen addition in the Tibetan grassland promoted loss of soil organic carbon but did not alter the bulk change of chemical structure

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Abstract. Nitrogen (N) and warming effects on ecosystem carbon (C) budgets and stabilization are critical to understand as C sequestration is considered as a mechanism to offset anthropogenic CO_2 emissions, which is important for accurately predicting ecosystem C sequestration and/or potential C loss, remaining controversial though. But the relevant information, especially for the intervention of environmental controls on grassland soil is limited in Tibetan plateau (TP) regions. Here we used a 9-year two-

way factorial experiment involving warming with open top chambers (+1.80 °C in the daytime and +0.77 °C in the nighttime at the soil surface) and multilevel nitrogen (N) enrichment treatments (0, 5, 10, and 15 g m^{-2} year^{-1}) in the TP to investigate the changes in SOC pool size and chemical structure. 9-year warming treatment significantly decreased SOC stock in the Tibetan grassland. We observed decreasing SOC concentrations which may be related to changes in the C degrading enzymes. Surprisingly, the SOC molecular structure remained unchanged in all N enrichment and warmed plots, suggesting that both treatments had affected all forms of SOC, from simple and complex polymeric in a similar way. Our results suggest that long-term warming stimulates soil C loss but no preference in SOC loss with different chemical structure.

Keywords: global warming, nitrogen deposition, SOC, molecular structure, C stabilization

1. Introduction

Soil organic matter is the largest organic carbon reservoir of near-surface terrestrial ecosystem (Dlamini et al. 2016). Even subtle acceleration in SOC decomposition will result in large CO_2 emissions (Davidson and Janssens 2006). So, knowledge of the factors affecting SOC storage and decomposition is essential for understanding the dynamically changing global C cycle. The influence of global warming on decomposition of soil carbon has been well documented (Poeplau et al. 2017, Guan et al. 2018, Ding et al. 2019b), but there remains considerable uncertainty in the potential response of soil C dynamics to the rapid global increase in reactive nitrogen (N, coming largely from agricultural fertilizers and fossil fuel combustion) as well as the combined effects with warming (Liang and Balser 2012, Devaraju et al. 2015, Li et al. 2017). For example, soil N availability would strongly affect microbial physiology and C-degrading enzymes (EnC), which can subsequently alter soil C feedbacks to warming (Mack *et al*. 2004; Contosta & Cooper 2015). EnC has been shown to play an important role in SOM nutrient cycling and catabolism (Chen et al. 2018a), and information on such activity can be used to investigate substrate nutrient demand and response to environmental changes (Allison et al. 2010; Wang et al. 2015). The knowledge gap demonstrated a need to focus research on biological and physicochemical controls of SOC stabilization and destabilization processes as a basis for understanding causal relationships and key processes that determine pool sizes and turnover rates of functional SOC pools (von Lützow and Kögel-Knabner 2009).

58   Soil warming experiments in the field have shown that warming generates a

59   considerable short-term soil C loss (Lu et al. 2013, Romero-Olivares et al. 2017). This

60   loss declines over time (e.g. > 2 years) (Romero-Olivares et al. 2017), although there is

61   evidence that it can continue for longer (e.g. > 20 years) (Melillo et al. 2017). Also,

62   indirect effects of warming on nutrient cycling (Pendall et al. 2004) or plant inputs

63   (Bradford et al. 2016) may have cascading effects on SOC quality and quantity (Lu et

64   al. 2013) and consequently on microbial decomposition of SOC, including recent plant-

65   derived material (Hicks Pries et al. 2017) or older SOC (Vaughn and Torn 2019).

66   Because ecosystems in alpine meadow are normally N limited (Hobbie et al. 2002),

67   increased N released from decomposing SOC could stimulate plant productivity,

68   thereby increasing ecosystem C storage (Moscatelli et al. 2008). However, field

69   evidence suggests that soil microbial activity and biomass may also be N limited in

70   some C-rich ecosystems (Mack et al. 2004, Rinnan et al. 2007). Therefore, increased N

71   released from decomposition of SOC could further fuel microbial activity and decrease

72   soil C storage. Besides, according to the priming effect hypothesis, the increase in N

73   availability and labile C substrates promotes microbial C utilization, thereby increasing

74   the degradation of less decomposable SOC and leading to a negative effect on soil C

75   accumulation over the long term (Riggs and Hobbie 2016). However, it has been proven

76   difficult to quantify bulk SOC stocks changes and organic matter composition directly

77   (Sistla et al. 2013, Van Gestel et al. 2018). As alternatives, molecular-level techniques

78   can detect how temperature affects plant and soil organic matter, microbial growth and

79   their community composition under climate warming (Feng et al. 2008, Xue et al. 2016,

80   Pold et al. 2017).

81   Since the molecular structure of organic material has long been thought to determine

82   long-term decomposition rates in soil humic substances, solid-state CPMAS ^{13}C NMR

83   and diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy has been

84   successfully applied in studies on changes of SOC chemical structure during organic

85   matter decomposition without any physical or chemical destruction (Schmidt et al.

86   2011). The structure of SOC could be very complex but by combining both techniques

87   (solid-state ^{13}C NMR and DRIFT) complementary information could be obtained on

88   aromatic and aliphatic components (Ferrari et al. 2011).

89   Despite the importance of the response of SOC stocks to warming and N enrichment

90   in the intact ecosystem, results about the chemical stabilization mechanisms (i.e.

91   molecular structure of SOC) in alpine meadows remained controversial. This

92   knowledge gap is significant because the Tibetan Plateau (TP) stores a large C pool,

93   with 36.6 Pg C stored in the top 3 m of the soil, accounting for 23.5% of China's total

94   organic soil-stored C and 2.5% of the global pool of soil C, which is of great importance

95   in regulating future global climate change and C emission (Genxu et al. 2002, Ding et

96   al. 2019a). At the same time, the TP has experienced climate warming at a rate that is

97   two times faster than that in other regions worldwide and is predicted to lead to great

98   soil C losses via microbial respiration in the future (Biskaborn et al. 2019). In addition,

99   during recent decades, the TP has been subject to high levels of N enrichment driven

100   by agricultural activities (up to ~8.0 g m^{-2} y^{-1}) (Gao et al. 2007, Bo et al. 2012, Zhang

101 and Fu 2020) and atmospheric N deposition (1 g N m^{-2} y^{-1}) (Lü and Tian 2007, Yu et al.

102 2019) with an annual rate of increase in deposition (0.053 g N m^{-2} y^{-1}) (Liu et al. 2013,

103 Wang et al. 2019b), and this kind of enrichment has been shown to induce soil C loss

104 and affect SOC stabilization in this typical N-constrained ecosystem (Xiao et al. 2021).

105 Since temperature is one of the main drivers of the vegetation growth and

106 decomposition of organic matter, on-going climate change may alter biophysical

107 processes with consequences for ecosystem functioning, especially in highly sensitive

108 cold regions such as the alpine meadow on the TP (Piao et al. 2006, Yang et al. 2008).

109 However, how and to what extent chemical stabilization of SOC shifts may occur, and

110 consequently SOC storage and C-climate feedback would respond to warming and N

111 enrichment in an alpine meadow ecosystem, remains largely unknown. Here, we used

112 soils from a 9-year experiment with a two-way factorial design involving soil warming

113 (daytime: 1.80°C; nighttime: 0.77°C) and control plots and N enrichments (0, 5, 10,

114 and 15 g m^{-2} y^{-1}; marked as N0, N1, N2, and N3, respectively) (Liu et al. 2016) on the

115 TP to examine the changes in the stock and molecular structure of SOC.

116 We hypothesized that 9-years N enrichment and warming would affect SOC stock and

117 the chemical structure of the SOC. N enrichment below a certain threshold may favor

118 C sequestration in the alpine grassland ecosystem but warming may result in the C loss.

119 And added N would stimulate hydrolytic enzyme activity while warming would repress

120 enzyme activity. Finally, we hypothesized that variation in enzyme response to N and

121 temperature would emerge as an important explanation for variability in the effect of

122 added N and warming on SOC stock.

123

2. Materials and methods

2.1 Site description

Plot sampling was conducted in a grassland ecosystem located on the eastern edge of the Tibetan Plateau, Maqu County, Gansu Province, China (101°53′ E, 35°58′ N, 3500 m above sea level, Figure 1 (NOAA 2015)), in August 2019. The grassland ecosystem of the TP covers an area of about 1.53 million km^2, accounting for nearly 60% of the total area of the TP (Liu et al., 2016). Alpine meadow is the main vegetation type in this area, the plant community is dominated by perennial herbaceous species of *Poaceae*, *Ranunculaceae*, and *Asteraceae*. The area of alpine meadow accounts for more than 44% of the area of alpine grasslands, and its SOC storage accounts for 56% of the SOC storage of alpine grasslands on the whole TP (Yang et al. 2008). The soil in the alpine meadow is classified as Mat-Cryic Cambisol (Hou et al. 2019). This region has a typical plateau continental climate. The mean annual precipitation is 620 mm, and most falls in the growing season (summer). The mean annual temperature is 1.2°C, with the lowest monthly mean temperature occurring in January (−10.7°C) and the highest monthly mean temperature occurring in July (11.7°C). During the past several decades, the mean annual temperatures in the region have risen at a rate of 0.58°C per decade (Liu et al. 2016).

2.2 Experimental design and soil sampling

A field-based warming experiment was established in June 2011 with a split-plot block design, in which both temperature (open-top chamber, +1.80°C in the daytime and +0.77°C in the nighttime at the soil surface) and nitrogen (0, 5, 10, and 15 g m^{-2} y^{-}

146   [1], corresponding to N0, N1, N2, and N3, respectively) were manipulated, with six

147   replicates per treatment (Liu et al., 2016). The 48 plots (8 treatments (N0, N1, N2, N3,

148   WN0, WN1, WN2, WN3) with 6 replicates each treatment) with roughly the same

149   species diversity and community structure were 5 × 5 m and were separated by 1 m

150   from adjacent edges. Additional details can be found in our previous studies (Sun et al.,

151   2023). Surface layer (0-10 cm) soils were collected from these 48 plots using a 4-cm-

152   diameter auger in August 2019. Then, the fresh soil samples were transported to the

153   laboratory on ice.

154   **2.3 Soil analysis**

155   Soil microbial biomass carbon (MBC) was measured according to the chloroform

156   fumigation extraction method using a TOC analyzer (Multi N/C 3100, Analytik Jena

157   GmbH, Germany) (Vance et al. 1987). The soil pH was determined in a 1:5 soil: water

158   suspension with a pH meter (PHS-3D, Rex, Shanghai, China). Bulk density samples

159   were dried at 105 °C for 48 h and calculated by dividing the oven-dried soil mass by

160   the steel cylinder volume (100 cm^3) because coarse fragments (stones or large roots)

161   were not obtained in ring samples. For soil organic carbon (SOC) analysis, air-dried

162   soil was ground and HCl-fumigated (Komada et al. 2008), and then the SOC

163   concentration was determined with an elemental analyzer (FlashSmart, Thermo Fisher

164   Scientific, USA). The SOC stocks (0-10 cm) were calculated by multiplying the SOC

165   concentration by the bulk density (Walter et al. 2016). At these 48 sites, all plants in

166   three plots (50 × 50 cm) were harvested and dried to determine the aboveground

167   biomass (AGB).

168   We measured the activity of four extracellular enzymes in the soil at an in situ pH

169   (Nie et al. 2013). The absorbance of the C degradation enzymes β-D-cellubiosidase

170   (CB), α-glucosidase (AG), β-glucosidase (BG) and β-xylosidase (XYL) were measured

171   using a Tecan infinite M200 microplate fluorometer (Grodig, Austria) with 365 nm

172   excitation and 460 nm emission filters. The activities were expressed in units of nmol

173    $h^{-1} g^{-1}$ dry soil. We combined CB, AG, BG and XYL into a C-degrading enzyme variable

174   (EnC).

175   **2.4 SOC molecular structure examination using NMR spectroscopy**

176   The soil samples used for NMR spectroscopy analysis were pretreated using HF (2%)

177   to eliminate paramagnetic materials, e.g. ferric ion and manganese ion, that may affect

178   the NMR signals (Skjemstad et al. 1994, Schmidt et al. 1997, Mathers et al. 2002). The

179   solid-state NMR spectra (^{13}C-CP-MAS) were recorded on a Bruker AVANCE III

180   600 MHz instrument (Bruker Instrument Inc., Billerica, MA, USA). The acquisition

181   conditions were set at frequency of 75.5 MHz, with 20 kHz spectra width, 5 kHz

182   spinning speed, 2 ms contact time, and 2.5 s recycle time. The regions of 0–210 ppm

183   spectra were plotted.

184   We examined seven chemical shift regions to represent the main C functional groups

185   (Golchin et al. 1997, Sun et al. 2019). We report proportions of each chemical shift area

186   and calculated 4 ratios indicative for the characteristics of soil organic matter. The alkyl

187   C, the most persistent fraction of SOC, comes from original plant biopolymers (such as

188   cutin, suberin and waxes) or from metabolic products of soil microorganisms (Ussiri

189   and Johnson 2003). As these materials decompose, the relative abundance of O-alkyl C

in the litter materials decreases, and there is a progressive increase in alkyl C
(Bonanomi et al. 2013). Therefore, the ratio of alkyl C to O-alkyl C (A/O-A = C_{0-45}/C_{60-90}) is an index represents the extent of SOC decomposition, the higher this ratio, the
higher the decomposition degree of SOC (Wang et al. 2015). Aromaticity ($C_{110-165}$/C_{0-165}), was used to indicate the complexity of molecular structure (Dai et al. 2001). The
ratio of aliphatic C/aromatic C (Alip/Arom), C_{0-110}/$C_{110-165}$, also indicates the molecular
structure of soil C, with higher Alip/Arom means less aromatic nuclear structure in
humus. The hydrophobic C/hydrophilic C (HB/HI) ratio, $(C_{0-45} + C_{110-165})$/$(C_{45-110} + C_{165-210})$, was used to reflect the stability of soil aggregation (Spaccini et al. 2006,
Wang et al. 2010). The higher values of HB/HI ratio indicated that SOC was more
hydrophobic (Cao et al., 2016), which, in turn, implied that SOC was more stable
(Spaccini et al., 2006, Wu et al., 2014).

2.5 Bulk soil organic matter composition using DRIFT spectroscopy

Because of the large number of variables affecting a spectrum, it is extremely
difficult to obtain a complete and fine molecular structure from a single spectrum
without additional knowledge obtained by other spectroscopic techniques (Ferrari et al.
2011). So, we employed another complementary molecular-level analysis called diffuse
reflectance infrared Fourier transform (DRIFT) spectroscopy, which is a useful method
for the characterization of organic matter (Olk et al. 2000) and humic substances (Mao
et al. 2008, Francioso et al. 2009), to explore potential shifts in SOC composition in
response to warming and N enrichment. To characterize warming/N-induced changes
in SOC composition, 6 mg of ground soil sample was examined by diffuse reflectance

infrared Fourier transform spectroscopy (DRIFT). Mid-infrared spectra were recorded

using a Bruker TENSOR 27 spectrometer (Billerica, Massachusetts, USA) from 4000

to 400 cm^{-1} (average of 16 scans per sample at 4 cm^{-1} resolution). Infrared absorption

bands were represented by functional groups. Infrared absorption bands were

represented by functional groups as follows: aliphatic C–H (2900 cm^{-1}), aromatic esters,

carbonyl/carboxyl C– –O (1735–1720 cm^{-1}), aromatic C– –C (1660–1600 cm^{-1}, 1430–

1380 cm^{-1}), lignin-like residues (1515–1500 cm^{-1}), phenolic/cellulose (1260–1210 cm^{-1}), and aromatic C–H (880, 805, 745 cm^{-1}) carbon (Niemeyer et al. 1992; Leifeld, 2006;

Chatterjee et al. 2012). A summary of the absorption bands associated with different

compound classes can be found in Figure S2. Additional details can be found in our

previous studies (Ofiti et al. 2021).

2.6 Statistical analysis

All data are presented as the mean values of six field replicates. Any significant

differences in soil physicochemical properties among the different N enrichment levels

and warming treatments were identified by using two-way ANOVA followed by

Tukey's HSD post hoc test, with differences considered to be statistically significant at

$P < 0.05$. The statistical analysis was conducted using SPSS 13.0 and R version 3.5.1

(R Foundation for Statistical Computing, Vienna, Austria, 2013). To access the direct

and indirect effects of external factors on SOC stock, structural equation modeling

(SEM) was performed using the R package 'plspm' and 'piecewiseSEM' (Li et al. 2020).

For this purpose, firstly, all data were tested for normality using the Kolmogorov–

Smirnov test, and the non-normal variables were log-transformed. Secondly, we

234   established a prior model based on prior knowledge of effects and relationships among

235   the driving factors. Finally, we selected the best model based on overall goodness of

236   fits, including the chi-square (χ^2) statistic, degrees of freedom (df), whole-model P

237   value, goodness of fit index, and the root-mean-square error of approximation

238   (Schermelleh-Engel et al. 2003).

239

3. Results

3.1 Bulk soil properties

N enrichment and warming have significant interaction on pH, AGB, EnC, C/N and SOC stock (P < 0.05, Figure 2). AGB, EnC and SOC stock increased significantly under N enrichment but the increment decreased with rising N addition concentration (P < 0.05, Figure 2b, 2c, 2f). Warming exacerbates soil acidification and decreased the AGB, EnC, MBC and the SOC stock significantly. Both N enrichment and warming significantly decreased C/N ratio (P < 0.05, Figure 2d). Except for MBC, the NW interactions significantly altered soil physicochemical properties (Figure 2, Table S1).

3.2 SOC speciation as seen by DRIFT and NMR spectroscopy

Changes in SOC molecular composition became apparent in diffuse reflectance infrared Fourier transform (DRIFT) and nuclear magnetic resonance (NMR) spectra (Figure 3, 4 and Figure S2, S3). In all N enrichment and warming treatments, there was a statistically non-significant change in the SOC composition and molecular structure observed by both DRIFT and NMR spectra. The relative abundance of carbonyl/carboxyl C=O, C=C aromatics compounds as well as lignin-like residues decreased slightly after N enrichment, not significantly though. The relative abundance of the phenolic/cellulose remained stable in all individual and interaction treatments (Figure 3 and S2).

The results of ^{13}C NMR spectroscopy indicated the relative abundance of different C components (Table 1, Figure 4 and S3), showing that the proportion of the seven C functional groups did not change in soils under N enrichment and warming treatments.

262 The relative proportions of the seven C functional groups were stable in the 8 treatments

263 in the following abundance order: O-alkyl C (mean 33%), followed by alkyl C (mean

264 22%), aromatic C (mean 12%), N-alkyl C (11%), carbonyl C and di-O-alkyl C (mean

265 8%), and finally phenolic C (mean 3%) (Table 1, Figure S3). The four indexes which

266 can represent the extent of SOC decomposition observed by NMR spectra also showed

267 no significant difference under all the N-enrichment and warming treatments (Figure

268 4), suggesting that SOC showed a similar degradation state at all N level enrichments

269 and warming treatments as well as the interaction effects.

270 **3.3 Factors driving the SOC stocks**

271 We then developed a structural equation model (SEM) to assess the direct and

272 indirect effects of soil variables on the SOC stocks (Figure 5). The SEM results revealed

273 strong connections among global change, biotic, and edaphic factors (Figure 5),

274 demonstrating a need to consider their interactions when predicting SOC stock and its

275 response to individual and interactive effects of N enrichment and warming. Overall,

276 the SEM explained 44%, 55% and 21% of the variance in SOC stock driven by N

277 enrichment, warming treatment and the interaction effects, respectively. In both N and

278 warming patterns, C-degrading enzymes showed an important indirect factor in

279 regulating SOC stock. N enrichment had a positive effect on SOC stock by enhancing

280 enzyme activities. In contrast, warming had a negative effect on SOC stock by

281 inhibiting microbial enzymes. Besides, warming had a strong negative direct effect on

282 SOC stock (Figure 5b). However, no significant direct or indirect pathways for the

283 interaction effects of N and warming on SOC stock were observed (Figure 5C).

284

4. Discussion

4.1. Effects of warming and N enrichment on soil C pool size

It is suggested that small N inputs can decrease CO_2 emissions by changing the interaction between plants and soil microbes in N-limited ecosystems, for example, by increasing plant productivity and root biomass and then organic C inputs to the soil by promoting N availability and thus retard litter and SOC decomposition (Franklin et al. 2003, Mo et al. 2008, Zhou et al. 2014). However, in an alpine grassland, Jiang et al. 2012 found that both plant growth and microbial activity were generally N-limited, but the ability of plants to capture soil inorganic N was much stronger than that of soil microorganisms. When N was added, increased N availability resulted in increased plant growth, microbial activity and plant biomass (Micks et al. 2004). Therefore, the decomposition of litter and SOM is enhanced by increasing the quantity of litter input or by elevating microbial activity, and consequently, soil functions would shift from C sequestration to C loss. The increased N has consequently reduced the soil pH by 0.26 globally in only one decade, which may significantly influence the microbial community composition and activity and then SOC sequestration capacity (Geisseler and Scow 2014, Tian and Niu 2015, Raza et al. 2021). This speculation is consistent with our results that N input below a threshold level (for example, 10 g m^{-2} in this study) may be beneficial for C sequestration in alpine meadows of the TP and can partly explain the patterns of SOC pool size under various N enrichment levels in this study. Specifically, the SOC stock increased following N enrichment, but as the N addition concentration increased, this growth progressively diminished, eventually even

disappearing. Our results revealed that alpine grassland ecosystems on the TP may become a potential C source under future scenarios of increasing N enrichment.

In our study presented here, the 9 years of warming resulted in a very significant SOC loss of 14 to 28 % (Figure 2 and S1). The TP stored large amounts of SOC because of the permafrost soil, where limited C decomposition has led to the accumulation of large SOC stocks (Hengl et al. 2014, Schuur et al. 2015). Previous studies showed that the vulnerability of soils with large C stocks derives from the high temperature sensitivity of C decomposition and biogeochemical restrictions on the processes driving soil C inputs (Davidson and Janssens 2006; García-Palacios et al. 2021). Contrast with that, in soils with low initial C stocks, small losses coming from accelerated decomposition induced by rising temperature may be offset by concurrent increases in plant growth and soil C stabilization (Day et al. 2008, Macias-Fauria et al. 2012, Crowther et al. 2015). However, in areas with larger SOC stocks, accelerated decomposition exceeds the potential C accumulation of plant growth, contributing to a significant C loss to the atmosphere (Luo et al. 2019).

4.2. Effects of N enrichment and warming on SOC chemical compositions

SOC chemical composition not only is controlled by the chemistry of the plant materials input to the soil, but also by the microbial processing and degradation of SOC (Baldock et al. 1992). Although N addition can stimulate plant growth and increase litter fall, it can also accelerate or slow down microbial processing of plant residues, thus altering the chemical composition of SOC (Wang et al. 2019a). Surprisingly we observed that the SOC molecular structure remained unchanged in all N enrichment

329   and warmed plots (Figure 3, 4).

330   As the predominant chemical component of SOC across all treatments in our study,

331   O-alkyl C is mainly composed of carbohydrates, peptides and other labile organic

332   components derived from the fresh material, which could be preferentially degraded

333   compared with more resistant components such as alkyl C (Simpson and Simpson, 2012,

334   He et al. 2018). So, we hypothesized that this result could indicate that N and warming

335   may have the same impact on the input of fresh plants on the TP. Unlike O-alkyl C,

336   lipids represent the main source of alkyl C (aliphatic chains), which is derived from

337   original plant biopolymers. Lignin and tannin represent the main source of aromatic C,

338   together with phenolic C, mainly originating from lignin and amino acids of peptides

339   (Baldock et al 1992). All these components are more resistant to microbial

340   decomposition than labile O-alkyl C (Simpson and Simpson, 2012). Our results

341   suggested that the proportions of the stable SOC chemical structures remained the same

342   between the different N enrichment concentrations and warming treatments, indicating

343   the synchronous degradation of SOC. The alkyl/O-alkyl ratio and aromaticity, normally

344   regarded as the indicator of the relative stage of SOC degradation and has been widely

345   used as an indicator to reflect the complexity of SOC chemical structure (Baldock et al.

346   1992), exhibited no significant difference among N enrichment or warming treatments,

347   suggesting that all N levels and warming treatments exerted similar effects on the

348   degradation of SOC and aromatic and complex molecular structure (Zhang et al. 2013).

349   Infrared spectroscopy of SOC showed a statistically non-significant change in the

350   treated plots and the control plots, which were consistent with a previous study that

351 showed the 4.5 years of +4 °C whole-soil warming did not change the relative

352 abundance of carbonyl/carboxyl C=O, and C=C aromatics compounds in the surface

353 soils (above 20 cm) from a forest (Ofiti et al. 2021). Collectively, the above results

354 suggested that molecular structure of surface SOC may not be as sensitive to long-term

355 warming as we thought before (Atanassova and Doerr 2011, Chen et al. 2018a). Surface

356 SOC is dominated by recent (less transformed) plant-litter inputs, which is less

357 degraded and transformed than subsoil SOC (Ofiti et al. 2021). The lack of change in

358 plant- and microorganism-derived organic matter in the surface soil may be due to slight

359 drying and warming near the surface (Soong et al. 2021) which could have inhibited or

360 resulted in relatively less surface inputs. We noticed that warming significantly reduced

361 AGB under N1 and N2 enrichments in this study (Figure 2). Compared with labile SOC,

362 stable SOC can be more vulnerable to priming once microbes are provided with

363 exogenous C substrates. This high vulnerability of stable SOC to priming warrants more

364 attention in future studies on SOC cycling and global change (Zhang et al. 2022).

365 Overall, the stabilized functional SOC molecular structure suggests that soil warming

366 and N enrichment had similarly affected the labile and stabilized SOC of this C-rich

367 grassland soil at the level of chemical stability of organic C molecules, along with the

368 C loss.

369 **4.3 Regulating factors of SOC stock**

370 Our interpretation that prolonged warming could reduce SOC storage is further

371 supported by the simultaneous reduction of different C pool sizes characterized with

372 various chemical structural complexity with long-term warming. Many previous studies

have shown that microorganisms preferentially use the labile C pool for community

utilization and turnover after short-term warming (Melillo et al. 2002, Kirschbaum

2004). However, after the initial microbial assimilation of readily accessible SOC with

warming, soil microorganisms can acclimate to C starvation through utilization of

chemical less available C with continued warming (Chen et al. 2020). This

transformation in microbial preference of C substrates can be facilitated by changes in

C-degrading enzyme activities (Crowther and Bradford 2013).

Our results indicated that C-degrading enzymes could play a key role in regulating

soil C storage (Figure 5a, 5b), which is in line with previous explanations for continued

soil C loss with long-term warming, such as shifts in microbial community and

physiology (Melillo et al. 2017, Metcalfe 2017), changes in microbial carbon use

efficiency (Tucker et al. 2013), and increased microbial accessibility to litter and SOC

(Doetterl et al. 2015, Bailey et al. 2019), which are all closely related to changes in

microbial C-degrading enzyme activities. For example, warming decreased the

abundance of lignin-derived compounds but increased ligninase activity in a mixed

temperate forest (Feng et al. 2008). Although only cellulase activity was measured in

our study, a previous meta-analysis study has shown significantly increasing ligninase

activity after warming, enhancing the evidence of microbial response to recalcitrant C

pools and the evidence of simultaneous loss of different C fractions after long-term

warming (Chen et al. 2018b). Microbial utilization of recalcitrant C pools could

substantially accelerate overall soil C loss. This is because depolymerization of these

recalcitrant macromolecules increases microbial accessibility to litter and SOC that was

395   protected by recalcitrant C pools before (Schmidt et al. 2011, Lehmann and Kleber 2015,

396   Paustian et al. 2016).

397   While N fertilization exerts both direct and indirect impacts on SOC, its influence on

398   carbonates is direct, leading to continuous losses. This not only serves as a source of

399   atmospheric CO_2 (Kim et al., 2020; Raza et al., 2020; Zamanian et al., 2018) but also

400   degrades soil structure and affects physical, chemical, and biological properties (Meng

401   and Li, 2019). Under acidic conditions, this process induces fundamental changes in

402   microbial community composition and enzyme activity critical for SOC stability

403   (Rowley et al., 2020). In ecosystems characterized by N limitation, such as permafrost

404   and peatland regions, N enrichment enhances N availability, accelerating the

405   decomposition of labile organic C, especially in these otherwise C-rich ecosystems.

406   This, in turn, results in decreased soil C availability (Craine et al., 2007; Janssens et al.,

407   2010; Song et al., 2017). A previous study at our research site revealed a significant

408   reduction in the soil labile C pool within the particulate organic C fraction with

409   increasing N enrichment, signifying a decline in soil C availability (Chen et al., 2019).

410   Our findings demonstrate that N enrichment significantly stimulates extracellular

411   enzyme (EnC) activities and enhances microbial demand for C (Figure 2), aligning with

412   prior research indicating that added N stimulates the activity of soil cellulose-degrading

413   enzymes (e.g., cellobiosidase (CB) and β-glucosidase (BG)) (Carreiro et al., 2000;

414   Saiya-Cork et al., 2002; Chen et al., 2017). This stimulation may be attributed to the

415   increase in C-acquiring enzymes resulting from heightened microbial demand for C,

416   especially in N-limited ecosystems (Keeler et al., 2009). Previous studies suggest that

417 N enrichment could induce C limitation by reducing plant allocation to fine root

418 production, leading to lower C input into the soil (Treseder, 2008). Thus, we propose

419 that factors beyond the thermal environment, such as N enrichment, can modulate soil

420 enzymes and alter substrate availability. Moreover, these processes can mediate the

421 strength of the soil C-climate feedback. Although N enrichment may increase soil C

422 sinks, this increase may be counteracted by warming. In summary, our results suggest

423 that warming and N enrichment have antagonistic interaction effects on SOC stock,

424 with differential effects on the contribution of alpine meadows to the soil C pool, which

425 may explain the result that the NW interaction did not show significant direct or indirect

426 effects on SOC storage in the SEM results. All these results underscore the importance

427 of considering soil C availability and enzymatic activity responses, which collectively

428 determine the response of the C balance to multiple environmental changes, for a more

429 comprehensive understanding of C storage dynamics.

430 **5. Conclusion**

431 Based on a 9-year warming (+1.80 °C in the daytime and +0.77 °C in the nighttime

432 at the soil surface) and different level N enrichment experiment (0, 5, 10, and 15 g m^{-2}

433 year^{-1}), we examined the responses of SOC stocks and their molecular components in

434 a Tibetan alpine meadow ecosystem. In summary, our results show little effects of soil

435 warming and N enrichment on the chemical composition of bulk soil despite ongoing

436 C loss in the warmed plots of the study site (Figure 2). The SOC molecular structure

437 suggested that the easily decomposable and stabilized SOC are synchronously affected

438 after 9-year warming and N treatments despite the large changes in SOC stocks. Given

439 the long residence time of some SOC (Schmidt et al., 2011), the similar loss of all

440 measurable chemical forms of SOC under global change treatments could have

441 important climate consequences. While we found little effects of soil warming on SOC

442 chemistry and molecular structure of bulk soil, consistent and long lasting changes

443 could appear with prolonged soil warming and decreasing SOC stocks in the following

444 years. In this process, the importance of enzyme activity must be emphasized, which

445 has been found to be an important indirect factor in regulating changes in SOC stocks.

446

Data availability

The data that support the findings of this study and those not presented within the article and its Supplementary Information file are available from https://doi.org/ 10.5281/zenodo.8289311.

Author contributions

M.N. developed the original ideas presented in the manuscript; H.S. performed the overall analysis with the assistance from Jintao Li. and Jinquan Li, N. O.; X.L. and S.Z. organized the field experiment; H.S., M.S. and M.N. wrote the first draft, and all authors jointly revised the manuscript.

Competing interests

The contact author has declared that none of the authors has any competing interests.

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References

Allison, S. D., Wallenstein, M. D., Bradford, M. A: Soil-carbon response to warming dependent on microbial physiology. Nat. Geosci. 3, 336-340, https://doi.org/10.1038/ngeo846, 2010.

Atanassova, I., and S. Doerr: Changes in soil organic compound composition associated with heat-induced increases in soil water repellency, Eur. J. Soil Sci., 62, 516-532, https://doi.org/10.1111/j.1365-2389.2011.01350.x, 2011.

Bailey, V. L., C. H. Pries, and K. Lajtha: What do we know about soil carbon destabilization? ENVIRON. RES. LETT., 14, https://doi.org/10.1088/1748-9326/ab2c11, 2019.

Baldock, J., J. Oades, A. Waters, X. Peng, A. Vassallo, and M. Wilson: Aspects of the chemical structure of soil organic materials as revealed by solid-state13C NMR spectroscopy. Biogeochemistry 16, 1-42, https://doi.org/10.1007/BF00024251, 1992.

Biskaborn, B. K., S. L. Smith, J. Noetzli, H. Matthes, G. Vieira, D. A. Streletskiy, P. Schoeneich, V. E. Romanovsky, A. G. Lewkowicz, A. Abramov, M. Allard, J. Boike, W. L. Cable, H. H. Christiansen, R. Delaloye, B. Diekmann, D. Drozdov, B. Etzelmüller, G. Grosse, M. Guglielmin, T. Ingeman-Nielsen, K. Isaksen, M. Ishikawa, M. Johansson, H. Johannsson, A. Joo, D. Kaverin, A. Kholodov, P. Konstantinov, T. Kröger, C. Lambiel, J.-P. Lanckman, D. Luo, G. Malkova, I. Meiklejohn, N. Moskalenko, M. Oliva, M. Phillips, M. Ramos, A. B. K. Sannel, D. Sergeev, C. Seybold, P. Skryabin, A. Vasiliev, Q. Wu, K. Yoshikawa, M. Zheleznyak, and H. Lantuit: Permafrost is warming at a global scale. Nat. Commun., 10, 264, https://doi.org/10.1038/s41467-018-08240-4, 2019.

Bonanomi, G., G. Incerti, F. Giannino, A. Mingo, V. Lanzotti, and S. Mazzoleni: Litter quality assessed by solid state 13C NMR spectroscopy predicts decay rate better than C/N and Lignin/N ratios.

490    Soil Biol. Biochem., 56, 40-48, https://doi.org/10.1016/j.soilbio.2012.03.003, 2013.

491 Bradford, M. A., W. R. Wieder, G. B. Bonan, N. Fierer, P. A. Raymond, and T. W. Crowther: Managing

492    uncertainty in soil carbon feedbacks to climate change. Nat. Clim. Change, 6, 751-758,

493    https://doi.org/10.1038/nclimate3071, 2016.

494 Cao, Z. Y., Wang, Y, Li, J, Zhang, J. J and He, N. P: Soil organic carbon contents, aggregate stability, and

495    humic acid composition in different alpine grasslands in Qinghai-Tibet Plateau. J. MT. SCI., 13,

496    2015-2027, https://doi.org/10.1007/s11629-015-3744-y, 2016.

497 Carreiro, M., Sinsabaugh, R., Repert, D. & Parkhurst, D. Microbial enzyme shifts explain litter decay

498    responses to simulated nitrogen deposition. Ecology **81**, 2359-2365,

499    https://doi.org/10.1890/0012-9658, 2000.

500 Chatterjee, S., Santos, F., Abiven, S., Itin, B., Stark, R.E., Bird, J.A: Elucidating the chemical structure

501    of pyrogenic organic matter by combining magnetic resonance, mid-infrared spectroscopy and

502    mass spectrometry. ORG. GEOCHEM., 51, 35–44,

503    https://doi.org/10.1016/j.orggeochem.2012.07.006, 2012.

504 Chen, H., Z. Yang, R. K. Chu, N. Tolic, L. Liang, D. E. Graham, S. D. Wullschleger, and B. Gu: Molecular

505    insights into Arctic soil organic matter degradation under warming. ENVIRON. SCI.

506    TECHNOL., 52, 4555-4564, https://doi.org/10.1021/acs.est.7b05469, 2018a.

507 Chen, J., L. Elsgaard, K. J. van Groenigen, J. E. Olesen, Z. Liang, Y. Jiang, P. E. Laerke, Y. Zhang, Y.

508    Luo, B. A. Hungate, R. L. Sinsabaugh, and U. Jorgensen: Soil carbon loss with warming: New

509    evidence from carbon-degrading enzymes. Global Change Biol., 26, 1944-1952,

510    https://doi.org/10.1111/gcb.14986, 2020.

511 Chen, J., Y. Luo, P. García-Palacios, J. Cao, M. Dacal, X. Zhou, J. Li, J. Xia, S. Niu, H. Yang, S.

Shelton, W. Guo, and K. J. van Groenigen: Differential responses of carbon-degrading enzyme activities to warming: Implications for soil respiration. Global Change Biol., 24, 4816-4826, https://doi.org/10.1111/gcb.14394, 2018b.

Chen, J., Luo, Y., Li, J., Zhou, X., Cao, J., Wang, R.-W., Wang, Y., Shelton, S., Jin, Z., Walker, L.M., Feng, Z., Niu, S., Feng, W., Jian, S. and Zhou, L: Costimulation of soil glycosidase activity and soil respiration by nitrogen addition. Glob. Change Biol. **23**, 1328-1337, https://doi.org/10.1111/gcb.13402, 2017.

Contosta, A. R., Frey, S. D., Cooper, A. B: Soil microbial communities vary as much over time as with chronic warming and nitrogen additions. Soil Biol. Biochem. **88**, 19-24, https://doi.org/10.1016/j.soilbio.2015.04.013, 2015.

Craine, J., Morrow, C. & Fierer, N: Microbial nitrogen limitation increase decomposition. Ecology **88**, 2105-2113, https://doi.org/10.1890/06-1847.1, 2007. Chen, Y., Liu, X., Hou, Y., Zhou, S., Zhu, B: Particulate organic carbon is more vulnerable to nitrogen addition than mineral-associated organic carbon in soil of an alpine meadow. Plant Soil, **458**, 1-11, https://doi.org/10.1007/s11104-019-04279-4, 2019.

Crowther, T. W., and M. A. Bradford: Thermal acclimation in widespread heterotrophic soil microbes. Ecol. Lett., 16, 469-477, https://doi.org/10.1111/ele.12069, 2013.

Crowther, T. W., S. M. Thomas, D. S. Maynard, P. Baldrian, K. Covey, S. D. Frey, L. T. van Diepen, and M. A. Bradford: Biotic interactions mediate soil microbial feedbacks to climate change. P. Nalt. Acad. Sci. USA, 112:7033-7038, https://doi.org/10.1073/pnas.1502956112, 2015.

Dai, K. O. H., C. E. Johnson, and C. T. Driscoll: Organic matter chemistry and dynamics in clear-cut and

533   unmanaged   hardwood   forest   ecosystems.   Biogeochemistry,   54,   51-83,

534   https://doi.org/10.1023/A:1010697518227, 2001.

535   Davidson, E. A., and I. A. Janssens: Temperature sensitivity of soil carbon decomposition and feedbacks

536   to climate change. Nature, 440, 165-173, https://doi.org/10.1038/nature04514, 2006.

537   Day, T. A., C. T. Ruhland, and F. S. Xiong: Warming increases aboveground plant biomass and C stocks

538   in vascular-plant-dominated Antarctic tundra. Global Change Biol., 14, 1827-1843,

539   https://doi.org/10.1111/j.1365-2486.2008.01623.x, 2008.

540   Devaraju, N., G. Bala, K. Caldeira, and R. Nemani: A model based investigation of the relative

541   importance of CO_2-fertilization, climate warming, nitrogen deposition and land use change on

542   the global terrestrial carbon uptake in the historical period. Clim. Dynam., 47, 173-190,

543   https://doi.org/10.1007/s00382-015-2830-8, 2015.

544   Ding, J., T. Wang, S. Piao, P. Smith, G. Zhang, Z. Yan, S. Ren, D. Liu, S. Wang, S. Chen, F. Dai, J. He,

545   Y. Li, Y. Liu, J. Mao, A. Arain, H. Tian, X. Shi, Y. Yang, N. Zeng, and L. Zhao: The paleoclimatic

546   footprint in the soil carbon stock of the Tibetan permafrost region. Nat. Commun., 10, 4195,

547   https://doi.org/10.1038/s41467-019-12214-5, 2019a.

548   Ding, X., S. Chen, B. Zhang, C. Liang, H. He, and W. R. Horwath: Warming increases microbial residue

549   contribution to soil organic carbon in an alpine meadow. Soil Biol. Biochem., 135, 13-19,

550   https://doi.org/10.1016/j.soilbio.2019.04.004, 2019b.

551   Dlamini, P., P. Chivenge, and V. Chaplot: Overgrazing decreases soil organic carbon stocks the most

552   under dry climates and low soil pH: A meta-analysis shows. Agr. Ecosyst. Environ., 221, 258-

553   269, https://doi.org/10.1016/j.agee.2016.01.026, 2016.

554   Doetterl, S., A. Stevens, J. Six, R. Merckx, K. Van Oost, M. Casanova Pinto, A. Casanova-Katny, C.

555   Muñoz, M. Boudin, and E. Zagal Venegas: Soil carbon storage controlled by interactions

556   between geochemistry and climate. Nat. Geosci., 8, 780-783, https://doi.org/10.1038/ngeo2516,

557   2015.

558   Feng, X., A. J. Simpson, K. P. Wilson, D. Dudley Williams, and M. J. Simpson: Increased cuticular

559   carbon sequestration and lignin oxidation in response to soil warming. Nat. Geosci., 1, 836-839,

560   https://doi.org/10.1038/ngeo361, 2008.

561   Ferrari, E., O. Francioso, S. Nardi, M. Saladini, N. D. Ferro, and F. Morari: DRIFT and HR MAS NMR

562   characterization of humic substances from a soil treated with different organic and mineral

563   fertilizers. J. Mol. Struct., 998, 216-224, https://doi.org/10.1016/j.molstruc.2011.05.035, 2011.

564   Francioso, O., D. Montecchio, P. Gioacchini, L. Cavani, C. Ciavatta, O. Trubetskoj, and O. Trubetskaya:

565   Structural differences of Chernozem soil humic acids SEC–PAGE fractions revealed by thermal

566   (TG–DTA) and spectroscopic (DRIFT) analyses. Geoderma, 152, 264-268,

567   https://doi.org/10.1016/j.geoderma.2009.06.011, 2009.

568   Franklin, O., P. Högberg, A. Ekblad, and G. I. Ågren: Pine forest floor carbon accumulation in response

569   to N and PK additions: bomb 14C modelling and respiration studies. Ecosystems, 6, 644-658,

570   https://doi.org/10.1007/s10021-002-0149-x, 2003.

571   Gao, Y., P. Luo, N. Wu, S. Yi, and H. Chen: Biomass and nitrogen responses to grazing intensity in an

572   alpine meadow on the eastern Tibetan Plateau. Pol. J. Ecol., 55 (3), 469-479,

573   https://doi.org/10.1007/BF02557215, 2007.

574   García-Palacios, P., T. W. Crowther, M. Dacal, I. P. Hartley, S. Reinsch, R. Rinnan, J. Rousk, J. van den

575   Hoogen, J.-S. Ye, and M. A. Bradford: Evidence for large microbial-mediated losses of soil

576   carbon under anthropogenic warming. Nat Rev Earth Environ., 2, 507–517,

577   https://doi.org/10.1038/s43017-021-00178-4, 2021.

578   Geisseler, D., and K. Scow: Long-term effects of mineral fertilizers on soil microorganisms – A review.

579   Soil Biol. Biochem., 75, 54–63, https://doi.org/10.1016/j.soilbio.2014.03.023, 2014.

580   Genxu, W., Q. Ju, C. Guodong, and L. Yuanmin: Soil organic carbon pool of grassland soils on the

581   Qinghai-Tibetan Plateau and its global implication. Sci. Total Environ., 291, 207-217,

582   https://doi.org/10.1016/S0048-9697(01)01100-7, 2002.

583   Golchin, A., P. Clarke, J. A. Baldock, T. Higashi, J. O. Skjemstad, and J. M. Oades: The effects of

584   vegetation and burning on the chemical composition of soil organic matter in a volcanic ash soil

585   as shown by 13C NMR spectroscopy. I. Whole soil and humic acid fraction. Geoderma, 76,

586   155-174, https://doi.org/10.1016/S0016-7061(96)00104-8, 1997.

587   Guan, S., N. An, N. Zong, Y. He, P. Shi, J. Zhang, and N. He: Climate warming impacts on soil organic

588   carbon fractions and aggregate stability in a Tibetan alpine meadow. Soil Biol. Biochem., 116,

589   224-236, https://doi.org/10.1016/j.soilbio.2017.10.011, 2018.

590   He, Y., X. He, M. Xu, W. Zhang, X. Yang, and S. Huang: Long-term fertilization increases soil organic

591   carbon and alters its chemical composition in three wheat-maize cropping sites across central

592   and   south   China.   Soil   and   Tillage   Research   177,   79-87,

593   https://doi.org/10.1016/j.still.2017.11.018, 2018.

594   Hengl, T., J. M. de Jesus, R. A. MacMillan, N. H. Batjes, G. B. Heuvelink, E. Ribeiro, A. Samuel-Rosa,

595   B. Kempen, J. G. Leenaars, and M. G. Walsh: SoilGrids1km—global soil information based on

596   automated mapping. PLoS ONE, 9, e105992, https://doi.org/10.1371/journal.pone.0105992,

597   2014.

598   Hicks Pries, C. E., C. Castanha, R. C. Porras, and M. S. Torn: The whole-soil carbon flux in response to

599   warming. Science, 355, 1420, https://doi.org/10.1126/science.aal1319, 2017.

600   Hobbie, S. E., T. A. Miley, and M. S. Weiss: Carbon and nitrogen cycling in soils from acidic and

601   nonacidic tundra with different glacial histories in Northern Alaska. Ecosystems, 5, 0761-0774,

602   https://doi.org/10.1007/s10021-002-0185-6, 2002.

603   Hou, Y., Y. Chen, X. Chen, K. He, and B. Zhu: Changes in soil organic matter stability with depth in two

604   alpine   ecosystems   on   the   Tibetan   Plateau.   Geoderma,   351,   153-162,

605   https://doi.org/10.1016/j.geoderma.2019.05.034, 2019.

606   Huang, Z., Z. Xu, C. Chen, and S. Boyd: Changes in soil carbon during the establishment of a hardwood

607   plantation   in   subtropical   Australia.   Forest   Ecol.   Manag.,   254,   46-55,

608   https://doi.org/10.1016/j.foreco.2007.07.021, 2008.

609   Janssens, I. A., Dieleman, W., Luyssaert, S., Subke, J. A., Reichstein, M., Ceulemans, R., Ciais, P.,

610   Dolman, A. J., Grace, J., Matteucci, G., Papale, D., Piao, S. L., Schulze, E. D., Tang, J., Law, B.

611   E: Reduction of forest soil respiration in response to nitrogen deposition. Nat. Geosci. **3**, 315-322,

612   https://doi.org/10.1038/ngeo844, 2010.

613   Jiang, C., Yu, G., Li, Y., Cao, G., Yang, Z., Sheng, W., Yu, W.,: Nutrient resorption of coexistence species

614   in alpine meadow of the Qinghai-Tibetan Plateau explains plant adaptation to nutrient-poor

615   environment. Ecol. Eng., 44, 1-9, https://doi.org/10.1016/j.ecoleng.2012.04.006, 2012.

616   Keeler, B.L., Hobbie, S.E. & Kellogg, L.E.J.E: Effects of long-term nitrogen addition on microbial

617   enzyme activity in eight forested and grassland sites: implications for litter and soil organic matter

618   decomposition. Ecosystems **12**, 1-15, https://doi.org/10.1007/s10021-008-9199-z, 2009.

619   Kim, JH., Jobbágy, EG., Richter, DD., Trumbore, SE., Jackson, RB: Agricultural acceleration of soil

620   carbonate weathering. Glob. Change Biol. **26**(10): 5988-6002, https://doi.org/10.1111/gcb.15207,

621   2020.

622   Kirschbaum, M. U.: Soil respiration under prolonged soil warming: are rate reductions caused by

623   acclimation or substrate loss? Global Change Biol., 10, 1870-1877,

624   https://doi.org/10.1111/j.1365-2486.2004.00852.x, 2004.

625   Komada, T., M. R. Anderson, and C. L. Dorfmeier: Carbonate removal from coastal sediments for the

626   determination of organic carbon and its isotopic signatures, δ^{13}C and Δ^{14}C: comparison of

627   fumigation and direct acidification by hydrochloric acid. Limnol. Oceanogr-Meth., 6, 254-262,

628   https://doi.org/10.4319/lom.2008.6.254, 2008.

629   Lehmann, J., and M. Kleber: The contentious nature of soil organic matter. Nature, 528, 60-68,

630   https://doi.org/10.1038/nature16069, 2015.

631   Leifeld, J: Application of diffuse reflectance FT-IR spectroscopy and partial leastsquares regression to

632   predict NMR properties of soil organic matter. Eur. J. Soil Sci., 57, 846–857,

633   https://doi.org/10.1111/j.1365-2389.2005.00776.x, 2006.

634   Li, B., He, Y., Wang, Z., Luo, P., Hong, Q. Decomposition process and scientific management of yak

635   dung on alpine meadow in the eastern Qinghai-Tibetan Plateau. Pratacultural Sci. 29 (8), 1302-

636   1306, 2012 (in Chinese abstract).

637   Li, J., M. Nie, E. Pendall, P. B. Reich, J. Pei, N. J. Noh, T. Zhu, B. Li, and C. Fang: Biogeographic

638   variation in temperature sensitivity of decomposition in forest soils. Global Change Biol., 26,

639   1873-1885, https://doi.org/10.1111/gcb.14838, 2020.

640   Li, Q., Y. Tian, X. Zhang, X. Xu, H. Wang, and Y. Kuzyakov: Labile carbon and nitrogen additions affect

641   soil organic matter decomposition more strongly than temperature. Appl. Soil Ecol., 114, 152-

160, https://doi.org/10.1016/j.apsoil.2017.01.009, 2017.

Liang, C., and T. C. Balser: Warming and nitrogen deposition lessen microbial residue contribution to soil carbon pool. Nat. Commun., 3, 1222, https://doi.org/10.1016/j.apsoil.2017.01.009, 2012.

Liu, X., S. Lyu, S. Zhou, and C. J. Bradshaw: Warming and fertilization alter the dilution effect of host diversity on disease severity. Ecology, 97, 1680-1689, https://doi.org/10.1890/15-1784.1, 2016.

Liu, X., Y. Zhang, W. Han, A. Tang, J. Shen, Z. Cui, P. Vitousek, J. W. Erisman, K. Goulding, P. Christie, A. Fangmeier, and F. Zhang: Enhanced nitrogen deposition over China. Nature, 494, 459-462, https://doi.org/10.1038/nature11917, 2013.

Lü, C. & Tian, H.: Spatial and temporal patterns of nitrogen deposition in China: Synthesis of observational data. J. Geophys. Res., 112, D22S05, https://doi.org/10.1029/2006JD007990, 2007.

Lu, M., X. Zhou, Q. Yang, H. Li, Y. Luo, C. Fang, J. Chen, X. Yang, and B. Li: Responses of ecosystem carbon cycle to experimental warming: a meta-analysis. Ecology, 94, 726-738, https://doi.org/10.1890/12-0279.1, 2013.

Luo, R., J. Fan, W. Wang, J. Luo, Y. Kuzyakov, J.-S. He, H. Chu, and W. Ding: Nitrogen and phosphorus enrichment accelerates soil organic carbon loss in alpine grassland on the Qinghai-Tibetan Plateau. Sci. Total Environ., 650: 303-312, https://doi.org/10.1016/j.scitotenv.2018.09.038, 2019.

Macias-Fauria, M., B. C. Forbes, P. Zetterberg, and T. Kumpula: Eurasian Arctic greening reveals teleconnections and the potential for structurally novel ecosystems. Nat. Clim. Change, 2, 613-618, https://doi.org/10.1038/nclimate1558, 2012.

Mack, M., E. Schuur, M. Bret-Harte, G. Shaver, and F. J. N. Chapin: Ecosystem carbon storage in arctic

tundra reduced by long-term nutrient fertilization. Nature, 431, 440-443, https://doi.org/10.1038/nature02887, 2004.

Mao, J., J. Yao, L. Wang, and W. Liu: Easily prepared high-quantum-yield CdS quantum dots in water using hyperbranched polyethylenimine as modifier. J. Colloid Interf. Sci., 319, 353-356, https://doi.org/10.1016/j.jcis.2007.10.027, 2008.

Mathers, N. J., Z. Xu, S. J. Berners-Price, M. S. Perera, and P. G. Saffigna: Hydrofluoric acid pre-treatment for improving ^{13}C CPMAS NMR spectral quality of forest soils in south-east Queensland, Australia. Soil Res., 40, 665-674, https://doi.org/10.1071/SR01073, 2002.

Melillo, J. M., S. D. Frey, K. M. Deangelis, W. J. Werner, M. J. Bernard, F. P. Bowles, G. Pold, M. A. Knorr, and A. S. Grandy: Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world. Science, 358, 101-105, https://doi.org/10.1126/science.aan2874, 2017.

Melillo, J. M., P. Steudler, J. D. Aber, K. Newkirk, H. Lux, F. Bowles, C. Catricala, A. Magill, T. Ahrens, and S. Morrisseau: Soil warming and carbon-cycle feedbacks to the climate system. Science, 298, 2173-2176, https://doi.org/10.1126/science.1074153, 2002.

Meng, J., Li, X.-A: Effects of carbonate on the structure and properties of loess and the corresponding mechanism: an experimental study of the Malan loess, Xi'an area, China. Bull. Eng. Geol. Environ. **78** (7), 4965–4976, https://doi.org/10.1007/s10064-018-01457-z, 2019.

Metcalfe, D. B.: Microbial change in warming soils. Science, 358, 41-42, https://doi.org/10.1126/science.aap7325, 2017.

Micks, P., Aber, J. D., Boone, R. D., Davidson, E. A.: Short-term soil respiration and nitrogen immobilization response to nitrogen applications in control and nitrogen-enriched temperate

forests. For. Ecol. Manage. 196, 57–70, https://doi.org/10.1016/j.foreco.2004.03.012, 2004.

Mo, J., W. Zhang, W. Zhu, P. Gundersen, Y. Fang, D. Li, and H. Wang: Nitrogen addition reduces soil respiration in a mature tropical forest in southern China. Global Change Biol., 14, 403-412, https://doi.org/10.1111/j.1365-2486.2007.01503.x, 2008.

Moscatelli, M. C., A. Lagomarsino, P. De Angelis, and S. Grego: Short-and medium-term contrasting effects of nitrogen fertilization on C and N cycling in a poplar plantation soil. Forest Ecol. Manag., 255, 447-454, https://doi.org/10.1016/j.foreco.2007.09.012, 2008.

Nie, M., E. Pendall, C. Bell, C. K. Gasch, S. Raut, S. Tamang, and M. D. Wallenstein: Positive climate feedbacks of soil microbial communities in a semi-arid grassland. Ecol. Lett., 16, 234-241, https://doi.org/10.1111/ele.12034, 2013.

Niemeyer, J., Chen, Y., Bollag, J.-M: Characterization of humic acids, composts, and peat by diffuse reflectance Fourier-transform infrared spectroscopy. Soil Sci. Soc. Am., 56, 135–140, https://doi.org/10.2136/sssaj1992.03615995005600010021x, 1992.

Ofiti, N. O. E., C. U. Zosso, J. L. Soong, E. F. Solly, M. S. Torn, G. L. B. Wiesenberg, and M. W. I. Schmidt: Warming promotes loss of subsoil carbon through accelerated degradation of plant-derived organic matter. Soil Biol. Biochem., 156, 108185, https://doi.org/10.1016/j.soilbio.2021.108185, 2021.

Olk, D. C., G. Brunetti, and N. Senesi: Decrease in Humification of Organic Matter with Intensified Lowland Rice Cropping A Wet Chemical and Spectroscopic Investigation. Soil Sci. Soc. Am. J., 64, 1337-1347, https://doi.org/10.2136/sssaj2000.6441337x, 2000.

Paustian, K., J. Lehmann, S. Ogle, D. Reay, G. P. Robertson, and P. Smith: Climate-smart soils. Nature, 532, 49-57, https://doi.org/10.1038/nature17174, 2016.

708 Pendall, E., S. Bridgham, P. J. Hanson, B. Hungate, D. W. Kicklighter, D. W. Johnson, B. E. Law, Y. Q.

709   Luo, J. P. Megonigal, M. Olsrud, M. G. Ryan, and S. Q. Wan: Below-ground process responses

710   to elevated CO2 and temperature: a discussion of observations, measurement methods, and

711   models. New Phytol., 162, 311-322, https://doi.org/10.1111/j.1469-8137.2004.01053.x, 2004.

712 Piao, S., J. Fang, and J. He: Variations in vegetation net primary production in the Qinghai-Xizang

713   Plateau, China, from 1982 to 1999. Climatic Change, 74, 253-267,

714   https://doi.org/10.1007/s10584-005-6339-8, 2006.

715 Poeplau, C., T. Kätterer, N. I. W. Leblans, and B. D. Sigurdsson: Sensitivity of soil carbon fractions and

716   their specific stabilization mechanisms to extreme soil warming in a subarctic grassland. Global

717   Change Biol., 23, 1316-1327, https://doi.org/10.1111/gcb.13491, 2017.

718 Pold, G., A. S. Grandy, J. M. Melillo, and K. M. DeAngelis: Changes in substrate availability drive

719   carbon cycle response to chronic warming. Soil Biol. Biochem., 110, 68-78,

720   https://doi.org/10.1016/j.soilbio.2017.03.002, 2017.

721 Raza, S., Miao, N.a., Wang, P., Ju, X., Chen, Z., Zhou, J., Kuzyakov, Y: Dramatic loss of inorganic carbon

722   by nitrogen-induced soil acidification in Chinese croplands. Glob. Change Biol. **26** (6), 3738–

723   3751, https://doi.org/10.1111/gcb.15101, 2020.

724 Raza, S., K. Zamanian, S. Ullah, Y. Kuzyakov, I. Virto, and J. Zhou: Inorganic carbon losses by soil

725   acidification jeopardize global efforts on carbon sequestration and climate change mitigation. J.

726   Clean. Prod., 315, 128036, https://doi.org/10.1016/j.jclepro.2021.128036, 2021.

727 Riggs, C. E., and S. E. Hobbie: Mechanisms driving the soil organic matter decomposition response to

728   nitrogen enrichment in grassland soils. Soil Biol. Biochem., 99, 54-65,

729   https://doi.org/10.1016/j.soilbio.2016.04.023, 2016.

730   Rinnan, R., A. Michelsen, E. BÅÅTh, and S. Jonasson: Fifteen years of climate change manipulations

731   alter soil microbial communities in a subarctic heath ecosystem. Global Change Biol., 13, 28-

732   39, https://doi.org/10.1111/j.1365-2486.2006.01263.x, 2007.

733   Romero-Olivares, A. L., S. D. Allison, and K. K. Treseder: Soil microbes and their response to

734   experimental warming over time: A meta-analysis of field studies. Soil Biol. Biochem., 107, 32-

735   40, https://doi.org/10.1016/j.soilbio.2016.12.026, 2017.

736   Rowley, M.C., Grand, S., Adatte, T., Verrecchia, E.P: A cascading influence of calcium carbonate on the

737   biogeochemistry and pedogenic trajectories of subalpine soils, Switzerland. Geoderma **361**,

738   114065, https://doi.org/10.1016/j.geoderma.2019.114065, 2020.

739   Saiya-Cork, K., Sinsabaugh, R. & Zak, D: The effects of long term nitrogen deposition on extracellular

740   enzyme activity in an Acer saccharum forest soil. Soil Biol. Biochem. **34**, 1309-1315,

741   https://doi.org/10.1016/S0038-0717(02)00074-3, 2002.

742   Schermelleh-Engel, K., H. Moosbrugger, and H. Müller: Evaluating the fit of structural equation models:

743   Tests of significance and descriptive goodness-of-fit measures. Methods of psychological

744   research online, 8, 23-74, 2003.

745   Schmidt, M., H. Knicker, P. G. Hatcher, and I. Kogel-Knabner: Improvement of ^{13}C and ^{15}N CPMAS

746   NMR spectra of bulk soils, particle size fractions and organic material by treatment with 10%

747   hydrofluoric acid. Eur. J. Soil Sci., 48, 319-328, https://doi.org/10.1111/j.1365-

748   2389.1997.tb00552.x, 1997.

749   Schmidt, M. W., M. S. Torn, S. Abiven, T. Dittmar, G. Guggenberger, I. A. Janssens, M. Kleber, I. Kögel-

750   Knabner, J. Lehmann, and D. A. Manning: Persistence of soil organic matter as an ecosystem

751   property. Nature, 478, 49-56, https://doi.org/10.1038/nature10386, 2011.

752   Schuur, E. A., A. D. McGuire, C. Schädel, G. Grosse, J. W. Harden, D. J. Hayes, G. Hugelius, C. D.

753   Koven, P. Kuhry, and D. M. Lawrence: Climate change and the permafrost carbon feedback.

754   Nature, 520, 171-179, https://doi.org/10.1038/nature14338, 2015.

755   Simpson, M. J., Simpson, A. J.: The chemical ecology of soil organic matter molecular constituents. J.

756   Chem. Ecol., 38, 768-784, https://doi.org/10.1007/s10886-012-0122-x, 2012.

757   Sistla, S. A., J. C. Moore, R. T. Simpson, L. Gough, G. R. Shaver, and J. P. Schimel: Long-term warming

758   restructures Arctic tundra without changing net soil carbon storage. Nature, 497, 615-618,

759   https://doi.org/10.1038/nature12129, 2013.

760   Skjemstad, J., P. Clarke, J. Taylor, J. Oades, and R. Newman: The removal of magnetic materials from

761   surface soils-a solid state ^{13}C CP/MAS NMR study. Soil Res., 32, 1215-1229,

762   https://doi.org/10.1071/SR9941215, 1994.

763   Song, Y., Song, C., Meng, H., Swarzenski, CM., Wang, X., Tan, W: Nitrogen additions affect litter quality

764   and soil biochemical properties in a peatland of Northeast China. Ecol. Eng. **100**, 175-185,

765   https://doi.org/10.1016/j.ecoleng.2016.12.025, 2017.

766   Soong, J. L., C. Castanha, C. E. Hicks Pries, N. Ofiti, R. C. Porras, W. J. Riley, M. W. Schmidt, and M.

767   S. Torn: Five years of whole-soil warming led to loss of subsoil carbon stocks and increased

768   CO2 efflux. Sci. Adv., 7, eabd1343, https://doi.org/10.1126/sciadv.abd1343, 2021.

769   Spaccini, R., J. Mbagwu, P. Conte, and A. Piccolo: Changes of humic substances characteristics from

770   forested   to   cultivated   soils   in   Ethiopia.   Geoderma,   132,   9-19,

771   https://doi.org/10.1016/j.geoderma.2005.04.015, 2006.

772   Sun, H., J. Jiang, L. Cui, W. Feng, Y. Wang, and J. Zhang: Soil organic carbon stabilization mechanisms

773   in a subtropical mangrove and salt marsh ecosystems. Sci. Total Environ., 673, 502-510,

774   https://doi.org/10.1016/j.scitotenv.2019.04.122, 2019.

775   Sun, H., Chen, H., Li, J., Zhang, Y., Liu, X., Li, J., Nie, M.: Nitrogen enrichment enhances thermal

776   acclimation   of   soil   microbial   respiration.   Biogeochemistry,   162(3),   343-357,

777   https://doi.org/10.1007/s10533-023-01014-1, 2023.

778   Tian, D., and S. Niu: A global analysis of soil acidification caused by nitrogen addition. Environ. Res.

779   Lett., 10, 024019, https://doi.org/10.1088/1748-9326/10/2/024019, 2015.

780   Treseder, K. K: Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecol.

781   Lett. **11**, 1111-1120, https://doi.org/10.1111/j.1461-0248.2008.01230.x, 2008.

782   Tucker, C. L., J. Bell, E. Pendall, and K. Ogle: Does declining carbon-use efficiency explain thermal

783   acclimation   of   soil   respiration   with   warming?   Global   Change   Biol.,   19,   252-263,

784   https://doi.org/10.1111/gcb.12036, 2013.

785   Ussiri, D. A., and C. E. Johnson: Characterization of organic matter in a northern hardwood forest soil

786   by   13C   NMR   spectroscopy   and   chemical   methods.   Geoderma,   111,   123-149,

787   https://doi.org/10.1016/S0016-7061(02)00257-4, 2003.

788   Van Gestel, N., Z. Shi, K. J. Van Groenigen, C. W. Osenberg, L. C. Andresen, J. S. Dukes, M. J. Hovenden,

789   Y. Luo, A. Michelsen, and E. Pendall: Predicting soil carbon loss with warming. Nature, 554,

790   E4-E5, https://doi.org/10.1038/nature20150, 2018.

791   Vance, E. D., P. C. Brookes, and D. S. Jenkinson: An extraction method for measuring soil microbial

792   biomass C. Soil Biol. Biochem., 19, 703-707, https://doi.org/10.1016/0038-0717(87)90052-6,

793   1987.

794   Vaughn, L. J., and M. S. Torn: [14]C evidence that millennial and fast-cycling soil carbon are equally

795   sensitive to warming. Nat. Clim. Change, 9, 467-471, https://doi.org/10.1038/s41558-019-

796   0468-y, 2019.

797   von Lützow, M., and I. Kögel-Knabner: Temperature sensitivity of soil organic matter decomposition—

798   what do we know? Biol. Fert. Soils, 46, 1-15, https://doi.org/10.1007/s00374-009-0413-8, 2009.

799   Walter, K., Don, A., Tiemeyer, B. and Freibauer, A.: Determining Soil Bulk Density for Carbon Stock

800   Calculations: A Systematic Method Comparison. Soil Sci. Soc. Am. J., 80, 579-591,

801   https://doi.org/10.2136/sssaj2015.11.0407, 2016.

802   Wang, C., N. He, J. Zhang, Y. Lv, and L. Wang: Long-term grazing exclusion improves the composition

803   and stability of soil organic matter in Inner Mongolian grasslands. PLoS ONE, 10, e0128837,

804   https://doi.org/10.1371/journal.pone.0128837, 2015.

805   Wang, H., S.-R. Liu, J.-M. Mo, J.-X. Wang, F. Makeschin, and M. Wolff: Soil organic carbon stock and

806   chemical composition in four plantations of indigenous tree species in subtropical China. Ecol.

807   Res., 25, 1071-1079, https://doi.org/10.1007/s11284-010-0730-2, 2010.

808   Wang, J.-J., R. D. Bowden, K. Lajtha, S. E. Washko, S. J. Wurzbacher, and M. J. Simpson: Long-term

809   nitrogen addition suppresses microbial degradation, enhances soil carbon storage, and alters the

810   molecular composition of soil organic matter. Biogeochemistry, 142, 299-313,

811   https://doi.org/10.1007/s10533-018-00535-4, 2019a.

812   Wang, J., B. Song, F. Ma, D. Tian, Y. Li, T. Yan, Q. Quan, F. Zhang, Z. Li, B. Wang, Q. Gao, W. Chen,

813   and S. Niu: Nitrogen addition reduces soil respiration but increases the relative contribution of

814   heterotrophic component in an alpine meadow. Funct. Ecol., 33, 2239-2253,

815   https://doi.org/10.1111/1365-2435.13433, 2019b.

816   Wu, J., Lin, H, Meng, C, Jiang, P, and Fu, W: Effects of intercropping grasses on soil organic carbon and

817   microbial community functional diversity under Chinese hickory (Carya cathayensis Sarg.)

stands. SOIL RES., 52(6), 575-583, https://10.1071/SR14021, 2014.

Xiao, J., S. Dong, Z. Zhao, Y. Han, S. Li, H. Shen, and C. Ding: Stabilization of soil organic carbon in the alpine meadow is dependent on the nitrogen deposition level on the Qinghai-Tibetan Plateau. Ecol. Eng., 170, 106348, https://doi.org/10.1016/j.ecoleng.2021.106348, 2021.

Xue, K., M. M Yuan, Z. J Shi, Y. Qin, Y. Deng, L. Cheng, L. Wu, Z. He, J. D. Van Nostrand, and R. Bracho: Tundra soil carbon is vulnerable to rapid microbial decomposition under climate warming. Nat. Clim. Change, 6, 595-600, https://doi.org/10.1038/nclimate2940, 2016.

Yang, Y., J. Fang, Y. Tang, C. Ji, C. Zheng, J. He, and B. Zhu: Storage, patterns and controls of soil organic carbon in the Tibetan grasslands. Global Change Biol., 14, 1592-1599, https://doi.org/10.1111/j.1365-2486.2008.01591.x, 2008.

Yu, G., Y. Jia, N. He, J. Zhu, Z. Chen, Q. Wang, S. Piao, X. Liu, H. He, X. Guo, Z. Wen, P. Li, G. Ding, and K. Goulding: Stabilization of atmospheric nitrogen deposition in China over the past decade. Nat. Geosci., 12, 424-429, https://doi.org/10.1038/s41561-019-0352-4, 2019.

Zamanian, K., Zarebanadkouki, M., Kuzyakov, Y: Nitrogen fertilization raises CO_2 efflux from inorganic carbon: a global assessment. Glob. Change Biol. 24 (7), 2810–2817, https://doi.org/10.1111/gcb.14148, 2018.

Zak, D. R., Z. B. Freedman, R. A. Upchurch, M. Steffens, and I. Kögel-Knabner: Anthropogenic N deposition increases soil organic matter accumulation without altering its biochemical composition. Global Change Biol., 23, 933-944, https://doi.org/10.1111/gcb.13480, 2017.

Zhang, H. R., and G. Fu: Responses of plant, soil bacterial and fungal communities to grazing vary with pasture seasons and grassland types, northern Tibet. Land Degrad. Dev., 32: 1821-1832, https://doi.org/10.1002/ldr.3835, 2020.

840   Zhang, J., S. Dou, and X. Song: Effect of long-term combined nitrogen and phosphorus fertilizer

841   application on ^{13}C CPMAS NMR spectra of humin in a Typic Hapludoll of northeast China. Eur.

842   J. Soil Sci., 60, 966-973, https://doi.org/10.1111/j.1365-2389.2009.01191.x, 2009.

843   Zhang, Q., J. Feng, J. Li, C.-Y. Huang, Y. Shen, W. Cheng, and B. Zhu: A distinct sensitivity to priming

844   effect between labile and stable soil organic carbon. New Phytol., 237(1), 88-99,

845   https://doi.org/10.1111/nph.18458, 2022.

846   Zhang, T., Y. Li, S. X. Chang, P. Jiang, G. Zhou, J. Liu, and L. Lin: Converting paddy fields to Lei

847   bamboo (Phyllostachys praecox) stands affected soil nutrient concentrations, labile organic

848   carbon pools, and organic carbon chemical compositions. Plant Soil 367, 249-261,

849   https://doi.org/10.1007/s11104-012-1551-6, 2013.

850   Zhou, L., X. Zhou, B. Zhang, M. Lu, Y. Luo, L. Liu, and B. Li: Different responses of soil respiration

851   and its components to nitrogen addition among biomes: a meta-analysis. Global Change Biol.,

852   20, 2332-2343, https://doi.org/10.1111/gcb.12490, 2014.

853   Zhou, P., G. Pan, R. Spaccini, and A. Piccolo: Molecular changes in particulate organic matter (POM) in

854   a typical Chinese paddy soil under different long-term fertilizer treatments. Eur. J. Soil Sci., 61,

855   231-242, https://doi.org/10.1111/j.1365-2389.2009.01223.x, 2010.

856

 Figure and table legends

858 **Figure 1.** Elevation map of the studied sites (a, the pentagram refers to the sampling

859 point), photo of the alpine meadow (b) and the diagram of the warming treatment (open-

860 top chamber) (c).

861 **Figure 2.** N and warming-induced changes in the soil properties (mean ± SE, n = 6).

862 Control (white bar) and warmed plots (black bar) at four different levels of simulated

863 N deposition. N0, N1, N2, and N3 indicate N-enrichments of 0, 5, 10, and 15 g N m^{-2}

864 year^{-1}, respectively. Parameters are: Soil pH (a); AGB, aboveground biomass (b); EnC,

865 C-degrading enzymes (c); C/N, ratio of soil C concentration to N concentration (d);

866 MBC, microbial biomass carbo (e); SOC, soil organic carbon stock (f).

867 **Figure 3.** N and warming-induced changes in the relative abundance of different

868 functional groups identifiable by diffuse reflectance infrared Fourier transform (DRIFT)

869 spectroscopy in warmed and control plots (mean ± SE, n = 6). The spectral regions were

870 assigned to aromatic carbonyl/carboxyl C=O groups, aromatic C=C groups, lignin-like

871 residues, and cellulose/phenolic.

872 **Figure 4.** Four different SOC chemical structural complexity indexes (mean ± SE, n =

873 6) from solid-state ^{13}C CPMASNMR spectra of soil samples from different treatments.

874 A/O-A=Alkyl C/O-alkyl C; HB/HI = hydrophobic C/hydrophilic C; Alip/Arom =

875 aliphatic C/aromatic C.

876 **Figure 5.** The factors regulating the SOC stock under (a) N enrichment, (b) warming

877 treatment and (c)interactive effects of N and warming. In the structural equation model

878 (SEM) analysis**,** black arrows represent significant positive pathways, gray arrows

879 represent significant negative pathways, and gray dashed arrows indicate nonsignificant

880 pathways. Values next to the arrows represent standardized effect sizes with statistical

881 significance (*P < 0.05; **P < 0.01; ***P < 0.001). The thickness of the arrow

882 represents the standardized effect sizes. C-degrading enzyme indicate sum of β-D-

883 cellubiosidase (CB), α-glucosidase (AG), β-glucosidase (BG) and β-xylosidase (XYL).

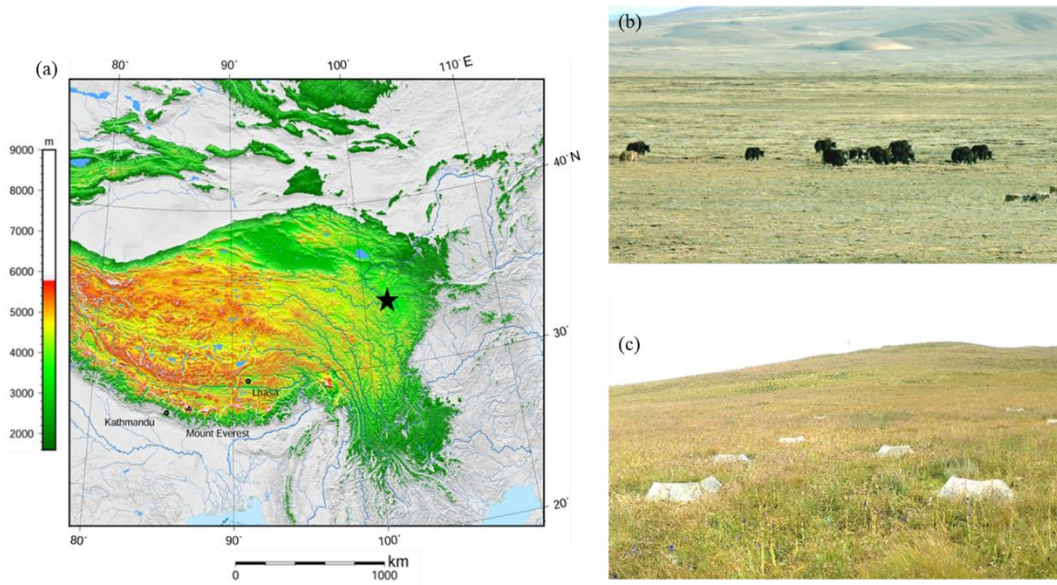
884 Goodness-of-fit statistics for the model are shown as follows: (a), χ^2 = 4.53, P = 0.53,

885 GFI =0.99, RMSEA < 0.001; (b), χ^2 = 4.47, P = 0.486, GFI = 0.99, RMSEA < 0.001.

886 **Table 1.** Relative intensities (mean ± SE, n = 6) of different carbon chemical shifts from

887 solid-state ^{13}C CPMAS NMR spectra of soil samples from N and warming treatments.

888

889 **Figure 1**

890

891   Note: the map (a) was cited from the Wikimedia Commons website (Tibet and

892   surrounding areas above 1600m, created using the Generic Mapping Tools)

893

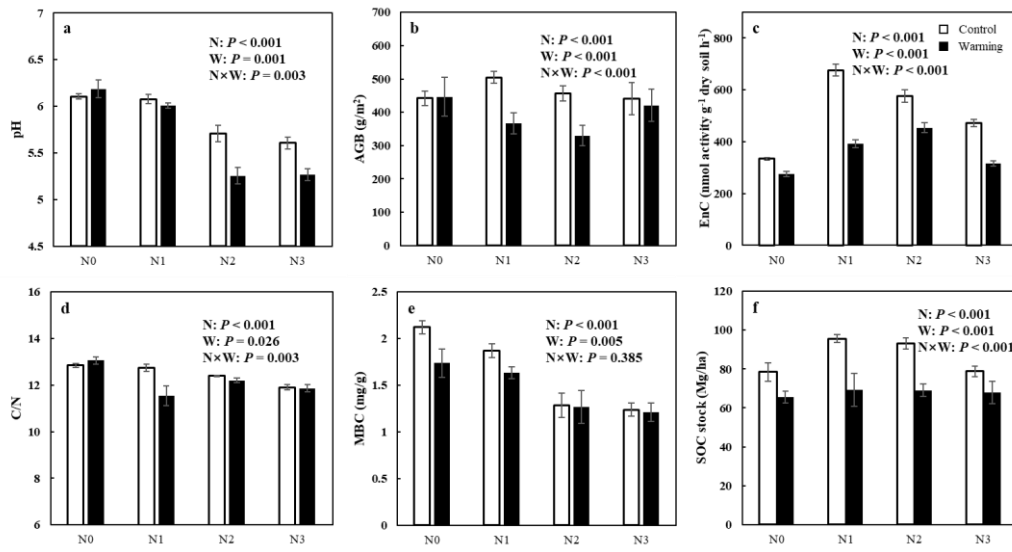
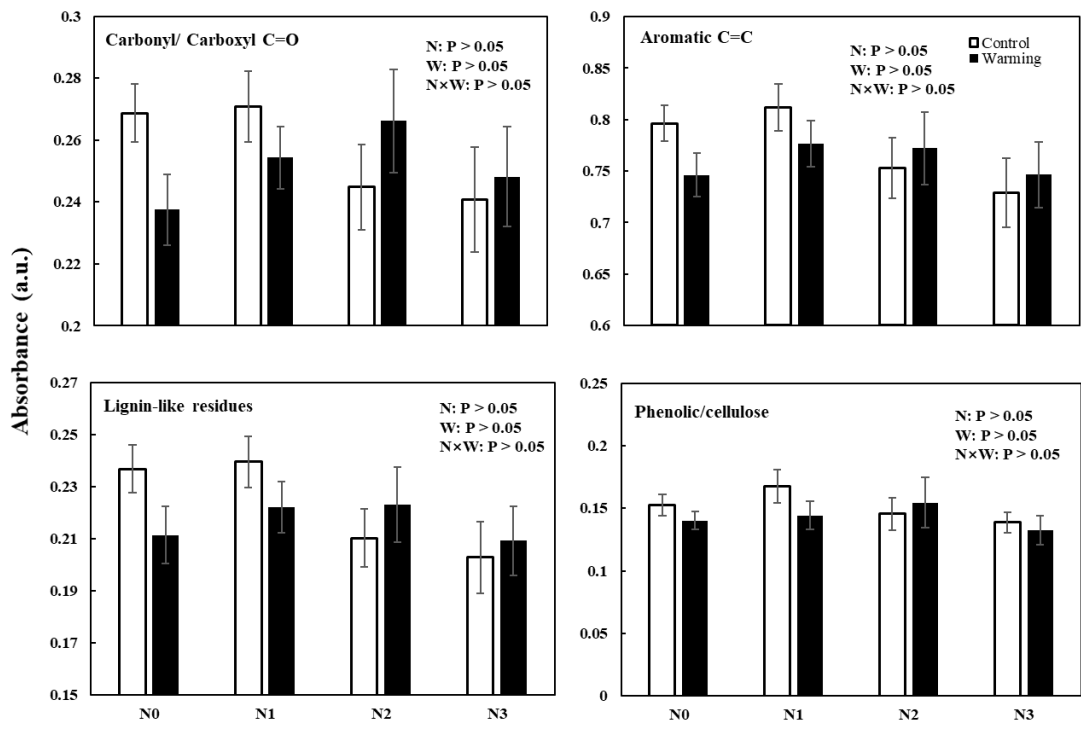



Figure 2

Figure 3

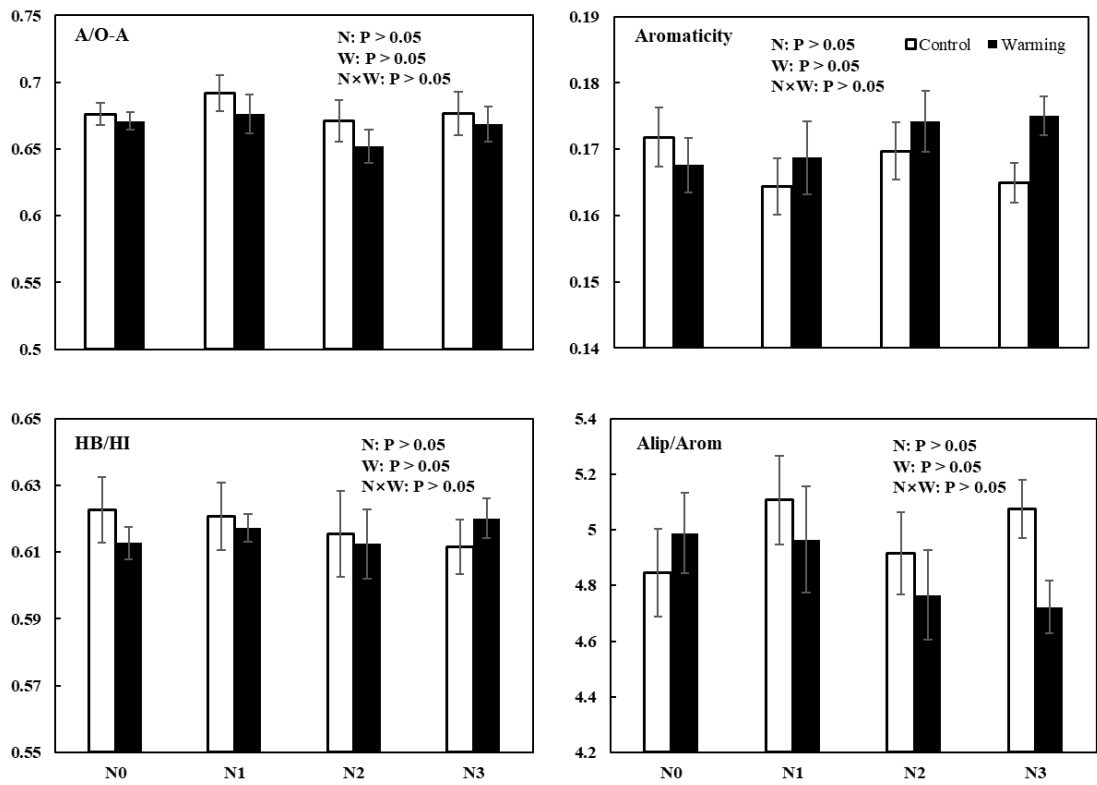
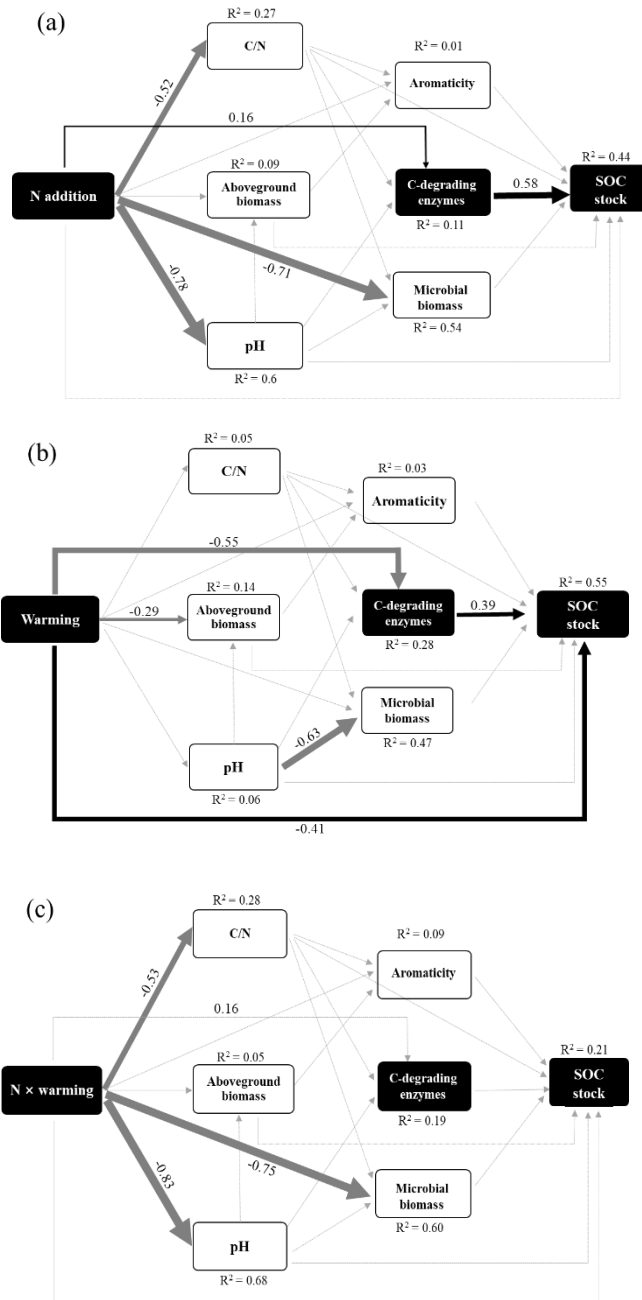
900 **Figure 4**

901
902

Figure 5

(a)

(b)

(c)

909 **Table 1**

Chemical shifts			Treatment			
			N0	N1	N2	N3
	Alkyl C (0-45 ppm)	C	22.64±0.15	23.22±0.17	22.55±0.2	22.85±0.32
		W	22.68±0.24	22.75±0.51	22.07±0.26	22.29±0.29
	N-alkyl C (45-60 ppm)	C	10.91±0.09	11.08±0.11	10.82±0.08	10.92±0.1
		W	10.73±0.06	10.6±0.24	10.54±0.08	10.67±0.16
	O-alkyl C (60-90 ppm)	C	33.5±0.35	33.62±0.53	33.67±0.54	33.83±0.47
		W	33.8±0.3	33.65±0.23	33.88±0.45	33.36±0.31
^{13}C NMR (%)	di-O-alkyl C (90-110 ppm)	C	8.74±0.09	8.65±0.09	8.9±0.13	8.82±0.12
		W	8.88±0.09	9.02±0.11	8.88±0.1	9.02±0.26
	Aromatic C (110-145 ppm)	C	12.25±0.33	11.62±0.26	12.05±0.3	11.45±0.28
		W	11.86±0.36	12.1±0.45	12.34±0.38	12.44±0.21
	Phenolic C (145-165 ppm)	C	3.47±0.1	3.44±0.11	3.47±0.08	3.64±0.05
		W	3.46±0.06	3.32±0.05	3.56±0.07	3.54±0.09
	Carbonyl C (165-210 ppm)	C	8.49±0.1	8.37±0.17	8.54±0.18	8.49±0.3
		W	8.61±0.16	8.57±0.24	8.73±0.14	8.68±0.19

910