1 **Nine years of warming and nitrogen addition in the Tibetan grassland**

2 **promoted loss of soil organic carbon but did not alter the bulk change**

3 **of chemical structure**

4 Huimin Sun[1, 2], Michael W.I. Schmidt[2], Jintao Li[1], Jinquan Li[1], Xiang Liu[3], Nicholas

5 O.E. Ofiti[2], Shurong Zhou[4], Ming Nie[1*]

6 1. Ministry of Education Key Laboratory for Biodiversity Science and Ecological Engineering,

7 National Observations and Research Station for Wetland Ecosystems of the Yangtze Estuary,

8 Institute of Biodiversity Science and Institute of Eco-Chongming, School of Life Sciences, Fudan

9 University, Shanghai, 200433, China.

10 2. Department of Geography, University of Zurich, Zurich, Switzerland.

11 3. State Key Laboratory of Grassland Agro-Ecosystem, Institute of Innovation Ecology, Lanzhou

12 University, 222 Tianshui South Road, Lanzhou 730000, China.

13 4. Key Laboratory of Genetics and Germplasm Innovation of Tropical Special Forest Trees and

14 Ornamental Plants, Ministry of Education, College of Forestry, Hainan University, Haikou 570228,

15 China.

16 *Correspondence to*: Ming Nie (mnie@fudan.edu.cn)

17 **Abstract.** Nitrogen (N) and warming effects on ecosystem carbon (C) budgets and

18 stabilization are critical to understand as C sequestration is considered as a mechanism

19 to offset anthropogenic CO_2 emissions, which is important for accurately predicting

20 ecosystem C sequestration and/or potential C loss, remaining controversial though. But

21 the relevant information, especially for the intervention of environmental controls on

22 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).

Soil warming experiments in the field have shown that warming generates a considerable short-term soil C loss (Lu et al. 2013, Romero-Olivares et al. 2017). This loss declines over time (e.g. > 2 years) (Romero-Olivares et al. 2017), although there is evidence that it can continue for longer (e.g. > 20 years) (Melillo et al. 2017). Also, indirect effects of warming on nutrient cycling (Pendall et al. 2004) or plant inputs (Bradford et al. 2016) may have cascading effects on SOC quality and quantity (Lu et al. 2013) and consequently on microbial decomposition of SOC, including recent plant-derived material (Hicks Pries et al. 2017) or older SOC (Vaughn and Torn 2019). Because ecosystems in alpine meadow are normally N limited (Hobbie et al. 2002), increased N released from decomposing SOC could stimulate plant productivity, thereby increasing ecosystem C storage (Moscatelli et al. 2008). However, field evidence suggests that soil microbial activity and biomass may also be N limited in some C-rich ecosystems (Mack et al. 2004, Rinnan et al. 2007). Therefore, increased N released from decomposition of SOC could further fuel microbial activity and decrease soil C storage. Besides, according to the priming effect hypothesis, the increase in N availability and labile C substrates promotes microbial C utilization, thereby increasing the degradation of less decomposable SOC and leading to a negative effect on soil C accumulation over the long term (Riggs and Hobbie 2016). However, it has been proven difficult to quantify bulk SOC stocks changes and organic matter composition directly (Sistla et al. 2013, Van Gestel et al. 2018). As alternatives, molecular-level techniques 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

and Fu 2020) and atmospheric N deposition (1 g N m^{-2} y^{-1}) (Lü and Tian 2007, Yu et al. 2019) with an annual rate of increase in deposition (0.053 g N m^{-2} y^{-1}) (Liu et al. 2013, Wang et al. 2019b), and this kind of enrichment has been shown to induce soil C loss and affect SOC stabilization in this typical N-constrained ecosystem (Xiao et al. 2021).

Since temperature is one of the main drivers of the vegetation growth and decomposition of organic matter, on-going climate change may alter biophysical processes with consequences for ecosystem functioning, especially in highly sensitive cold regions such as the alpine meadow on the TP (Piao et al. 2006, Yang et al. 2008). However, how and to what extent chemical stabilization of SOC shifts may occur, and consequently SOC storage and C-climate feedback would respond to warming and N enrichment in an alpine meadow ecosystem, remains largely unknown. Here, we used soils from a 9-year experiment with a two-way factorial design involving soil warming (daytime: 1.80°C; nighttime: 0.77°C) and control plots and N enrichments (0, 5, 10, and 15 g m^{-2} y^{-1}; marked as N0, N1, N2, and N3, respectively) (Liu et al. 2016) on the TP to examine the changes in the stock and molecular structure of SOC.

We hypothesized that 9-years N enrichment and warming would affect SOC stock and the chemical structure of the SOC. N enrichment below a certain threshold may favor C sequestration in the alpine grassland ecosystem but warming may result in the C loss. And added N would stimulate hydrolytic enzyme activity while warming would repress enzyme activity. Finally, we hypothesized that variation in enzyme response to N and temperature would emerge as an important explanation for variability in the effect of added N and warming on SOC stock.

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

190 in the litter materials decreases, and there is a progressive increase in alkyl C

191 (Bonanomi et al. 2013). Therefore, the ratio of alkyl C to O-alkyl C ($A/O\text{-}A = C_{0-45}/C_{60-90}$)

192 is an index represents the extent of SOC decomposition, the higher this ratio, the

193 higher the decomposition degree of SOC (Wang et al. 2015). Aromaticity ($C_{110-165}/C_{0-165}$),

194 was used to indicate the complexity of molecular structure (Dai et al. 2001). The

195 ratio of aliphatic C/aromatic C (Alip/Arom), $C_{0-110}/C_{110-165}$, also indicates the molecular

196 structure of soil C, with higher Alip/Arom means less aromatic nuclear structure in

197 humus. The hydrophobic C/hydrophilic C (HB/HI) ratio, $(C_{0-45} + C_{110-165})/(C_{45-110} + C_{165-210})$,

198 was used to reflect the stability of soil aggregation (Spaccini et al. 2006,

199 Wang et al. 2010). The higher values of HB/HI ratio indicated that SOC was more

200 hydrophobic (Cao et al., 2016), which, in turn, implied that SOC was more stable

201 (Spaccini et al., 2006, Wu et al., 2014).

2.5 Bulk soil organic matter composition using DRIFT spectroscopy

203 Because of the large number of variables affecting a spectrum, it is extremely

204 difficult to obtain a complete and fine molecular structure from a single spectrum

205 without additional knowledge obtained by other spectroscopic techniques (Ferrari et al.

206 2011). So, we employed another complementary molecular-level analysis called diffuse

207 reflectance infrared Fourier transform (DRIFT) spectroscopy, which is a useful method

208 for the characterization of organic matter (Olk et al. 2000) and humic substances (Mao

209 et al. 2008, Francioso et al. 2009), to explore potential shifts in SOC composition in

210 response to warming and N enrichment. To characterize warming/N-induced changes

211 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}

^{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 showed a significant interaction effect for 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 N and warming 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

262   functional groups did not change in soils under N enrichment and warming treatments.

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

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

265   22%), aromatic C (mean 12%), N-alkyl C (11%), carbonyl C and di-O-alkyl C (mean

266   8%), and finally phenolic C (mean 3%) (Table 1, Figure S3). The four indexes which

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

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

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

270   and warming treatments as well as the interaction effects.

271   **3.3 Factors driving the SOC stocks**

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

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

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

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

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

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

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

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

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

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

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

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

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

285

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

308   disappearing. Our results revealed that alpine grassland ecosystems on the TP may

309   become a potential C source under future scenarios of increasing N enrichment.

310   In our study presented here, the 9 years of warming resulted in a very significant

311   SOC loss of 14 to 28 % (Figure 2 and S1). The TP stored large amounts of SOC because

312   of the permafrost soil, where limited C decomposition has led to the accumulation of

313   large SOC stocks (Hengl et al. 2014, Schuur et al. 2015). Previous studies showed that

314   the vulnerability of soils with large C stocks derives from the high temperature

315   sensitivity of C decomposition and biogeochemical restrictions on the processes driving

316   soil C inputs (Davidson and Janssens 2006; García-Palacios et al. 2021). Contrast with

317   that, in soils with low initial C stocks, small losses coming from accelerated

318   decomposition induced by rising temperature may be offset by concurrent increases in

319   plant growth and soil C stabilization (Day et al. 2008, Macias-Fauria et al. 2012,

320   Crowther et al. 2015). However, in areas with larger SOC stocks, accelerated

321   decomposition exceeds the potential C accumulation of plant growth, contributing to a

322   significant C loss to the atmosphere (Luo et al. 2019).

323   **4.2. Effects of N enrichment and warming on SOC chemical compositions**

324   SOC chemical composition not only is controlled by the chemistry of the plant

325   materials input to the soil, but also by the microbial processing and degradation of SOC

326   (Baldock et al. 1992). Although N addition can stimulate plant growth and increase

327   litter fall, it can also accelerate or slow down microbial processing of plant residues,

328   thus altering the chemical composition of SOC (Wang et al. 2019a). Surprisingly we

329   observed that the SOC molecular structure remained unchanged in all N enrichment

330   and warmed plots (Figure 3, 4).

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

347   1992), exhibited no significant difference among N enrichment or warming treatments,

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

369 C loss.

370 **4.3 Regulating factors of SOC stock**

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

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

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

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

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

376 2004). However, after the initial microbial assimilation of readily accessible SOC with

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

395 recalcitrant macromolecules increases microbial accessibility to litter and SOC that was

protected by recalcitrant C pools before (Schmidt et al. 2011, Lehmann and Kleber 2015, Paustian et al. 2016).

While N fertilization exerts both direct and indirect impacts on SOC, its influence on carbonates is direct, leading to continuous losses. This not only serves as a source of atmospheric CO_2 (Kim et al., 2020; Raza et al., 2020; Zamanian et al., 2018) but also degrades soil structure and affects physical, chemical, and biological properties (Meng and Li, 2019). Under acidic conditions, this process induces fundamental changes in microbial community composition and enzyme activity critical for SOC stability (Rowley et al., 2020). In ecosystems characterized by N limitation, such as alpine grasslands, N enrichment enhances N availability, accelerating the decomposition of labile organic C, especially in these otherwise C-rich ecosystems. This, in turn, results in decreased soil C availability (Craine et al., 2007; Janssens et al., 2010; Song et al., 2017). A previous study at our research site revealed a significant reduction in the soil labile C pool within the particulate organic C fraction with increasing N enrichment, signifying a decline in soil C availability (Chen et al., 2019). Our findings demonstrate that N enrichment significantly stimulates extracellular enzyme (EnC) activities and enhances microbial demand for C (Figure 2), aligning with prior research indicating that added N stimulates the activity of soil cellulose-degrading enzymes (e.g., cellobiosidase (CB) and β-glucosidase (BG)) (Carreiro et al., 2000; Saiya-Cork et al., 2002; Chen et al., 2017). This stimulation may be attributed to the increase in C-acquiring enzymes resulting from heightened microbial demand for C, especially in N-limited ecosystems (Keeler et al., 2009). Previous studies suggest that N enrichment

could induce C limitation by reducing plant allocation to fine root production, leading

to lower C input into the soil (Treseder, 2008). Thus, we propose that factors beyond

the thermal environment, such as N enrichment, can modulate soil enzymes and alter

substrate availability. Moreover, these processes can mediate the strength of the soil C-

climate feedback. Although N enrichment may increase soil C sinks, this increase may

be counteracted by warming. In summary, our results suggest that warming and N

enrichment have antagonistic interaction effects on SOC stock, with differential effects

on the contribution of alpine meadows to the soil C pool, which may explain the result

that the N and warming interaction did not show significant direct or indirect effects on

SOC storage in the SEM results. All these results underscore the importance of

considering soil C availability and enzymatic activity responses, which collectively

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

comprehensive understanding of C storage dynamics.

5. Conclusion

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

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

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

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

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

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

suggested that the easily decomposable and stabilized SOC are synchronously affected

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

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

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

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

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

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

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

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

447

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.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (91951112 and 32101377), the Program of Shanghai Academic/Technology Research Leader (21XD1420700), the'Shuguang Program' supported by Shanghai Education Development Foundation and Shanghai Municipal Education Commission (21SG02), the Shanghai Pilot Program for Basic Research—Fudan University 21TQ1400100 (21TQ004), the Science and Technology Department of Shanghai (21DZ1201902), the Shanghai Pujiang Program (2020PJD003), and the Swiss National Science Foundtion SNF-project 172744 (DEEP C) awarded to M.W.I.S. and N.O.E.O..

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.

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

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

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

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

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

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

497   2015-2027, https://doi.org/10.1007/s11629-015-3744-y, 2016.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

513   Shelton, W. Guo, and K. J. van Groenigen: Differential responses of carbon-degrading

514   enzyme activities to warming: Implications for soil respiration. Global Change Biol., 24,

515   4816-4826, https://doi.org/10.1111/gcb.14394, 2018b.

516   Chen, J., Luo, Y., Li, J., Zhou, X., Cao, J., Wang, R.-W., Wang, Y., Shelton, S., Jin, Z., Walker, L.M.,

517   Feng, Z., Niu, S., Feng, W., Jian, S. and Zhou, L: Costimulation of soil glycosidase activity and

518   soil respiration by nitrogen addition. Glob. Change Biol. **23**, 1328-1337,

519   https://doi.org/10.1111/gcb.13402, 2017.

520   Contosta, A. R., Frey, S. D., Cooper, A. B: Soil microbial communities vary as much over time as with

521   chronic warming and nitrogen additions. Soil Biol. Biochem. **88**, 19-24,

522   https://doi.org/10.1016/j.soilbio.2015.04.013, 2015.

523   Craine, J., Morrow, C. & Fierer, N: Microbial nitrogen limitation increase decomposition. Ecology **88**,

524   2105-2113, https://doi.org/10.1890/06-1847.1, 2007. Chen, Y., Liu, X., Hou, Y., Zhou, S., Zhu, B:

525   Particulate organic carbon is more vulnerable to nitrogen addition than mineral-associated organic

526   carbon in soil of an alpine meadow. Plant Soil, **458**, 1-11, https://doi.org/10.1007/s11104-019-

527   04279-4, 2019.

528   Crowther, T. W., and M. A. Bradford: Thermal acclimation in widespread heterotrophic soil microbes.

529   Ecol. Lett., 16, 469-477, https://doi.org/10.1111/ele.12069, 2013.

530   Crowther, T. W., S. M. Thomas, D. S. Maynard, P. Baldrian, K. Covey, S. D. Frey, L. T. van Diepen, and

531   M. A. Bradford: Biotic interactions mediate soil microbial feedbacks to climate change. P. Nalt.

532   Acad. Sci. USA, 112:7033-7038, https://doi.org/10.1073/pnas.1502956112, 2015.

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

unmanaged hardwood forest ecosystems. Biogeochemistry, 54, 51-83, https://doi.org/10.1023/A:1010697518227, 2001.

Davidson, E. A., and I. A. Janssens: Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature, 440, 165-173, https://doi.org/10.1038/nature04514, 2006.

Day, T. A., C. T. Ruhland, and F. S. Xiong: Warming increases aboveground plant biomass and C stocks in vascular-plant-dominated Antarctic tundra. Global Change Biol., 14, 1827-1843, https://doi.org/10.1111/j.1365-2486.2008.01623.x, 2008.

Devaraju, N., G. Bala, K. Caldeira, and R. Nemani: A model based investigation of the relative importance of CO_2-fertilization, climate warming, nitrogen deposition and land use change on the global terrestrial carbon uptake in the historical period. Clim. Dynam., 47, 173-190, https://doi.org/10.1007/s00382-015-2830-8, 2015.

Ding, J., T. Wang, S. Piao, P. Smith, G. Zhang, Z. Yan, S. Ren, D. Liu, S. Wang, S. Chen, F. Dai, J. He, Y. Li, Y. Liu, J. Mao, A. Arain, H. Tian, X. Shi, Y. Yang, N. Zeng, and L. Zhao: The paleoclimatic footprint in the soil carbon stock of the Tibetan permafrost region. Nat. Commun., 10, 4195, https://doi.org/10.1038/s41467-019-12214-5, 2019a.

Ding, X., S. Chen, B. Zhang, C. Liang, H. He, and W. R. Horwath: Warming increases microbial residue contribution to soil organic carbon in an alpine meadow. Soil Biol. Biochem., 135, 13-19, https://doi.org/10.1016/j.soilbio.2019.04.004, 2019b.

Dlamini, P., P. Chivenge, and V. Chaplot: Overgrazing decreases soil organic carbon stocks the most under dry climates and low soil pH: A meta-analysis shows. Agr. Ecosyst. Environ., 221, 258-269, https://doi.org/10.1016/j.agee.2016.01.026, 2016.

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

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

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

558   2015.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

587   155-174, https://doi.org/10.1016/S0016-7061(96)00104-8, 1997.

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

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

590   224-236, https://doi.org/10.1016/j.soilbio.2017.10.011, 2018.

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

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

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

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

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

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

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

598   2014.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

carbonate weathering. Glob. Change Biol. **26**(10): 5988-6002, https://doi.org/10.1111/gcb.15207, 2020.

Kirschbaum, M. U.: Soil respiration under prolonged soil warming: are rate reductions caused by acclimation or substrate loss? Global Change Biol., 10, 1870-1877, https://doi.org/10.1111/j.1365-2486.2004.00852.x, 2004.

Komada, T., M. R. Anderson, and C. L. Dorfmeier: Carbonate removal from coastal sediments for the determination of organic carbon and its isotopic signatures, $\delta^{13}C$ and $\Delta^{14}C$: comparison of fumigation and direct acidification by hydrochloric acid. Limnol. Oceanogr-Meth., 6, 254-262, https://doi.org/10.4319/lom.2008.6.254, 2008.

Lehmann, J., and M. Kleber: The contentious nature of soil organic matter. Nature, 528, 60-68, https://doi.org/10.1038/nature16069, 2015.

Leifeld, J: Application of diffuse reflectance FT-IR spectroscopy and partial leastsquares regression to predict NMR properties of soil organic matter. Eur. J. Soil Sci., 57, 846–857, https://doi.org/10.1111/j.1365-2389.2005.00776.x, 2006.

Li, B., He, Y., Wang, Z., Luo, P., Hong, Q. Decomposition process and scientific management of yak dung on alpine meadow in the eastern Qinghai-Tibetan Plateau. Pratacultural Sci. 29 (8), 1302-1306, 2012 (in Chinese abstract).

Li, J., M. Nie, E. Pendall, P. B. Reich, J. Pei, N. J. Noh, T. Zhu, B. Li, and C. Fang: Biogeographic variation in temperature sensitivity of decomposition in forest soils. Global Change Biol., 26, 1873-1885, https://doi.org/10.1111/gcb.14838, 2020.

Li, Q., Y. Tian, X. Zhang, X. Xu, H. Wang, and Y. Kuzyakov: Labile carbon and nitrogen additions affect soil organic matter decomposition more strongly than temperature. Appl. Soil Ecol., 114, 152-

643   160, https://doi.org/10.1016/j.apsoil.2017.01.009, 2017.

644   Liang, C., and T. C. Balser: Warming and nitrogen deposition lessen microbial residue contribution to

645   soil carbon pool. Nat. Commun., 3, 1222, https://doi.org/10.1016/j.apsoil.2017.01.009, 2012.

646   Liu, X., S. Lyu, S. Zhou, and C. J. Bradshaw: Warming and fertilization alter the dilution effect of host

647   diversity on disease severity. Ecology, 97, 1680-1689, https://doi.org/10.1890/15-1784.1, 2016.

648   Liu, X., Y. Zhang, W. Han, A. Tang, J. Shen, Z. Cui, P. Vitousek, J. W. Erisman, K. Goulding, P. Christie,

649   A. Fangmeier, and F. Zhang: Enhanced nitrogen deposition over China. Nature, 494, 459-462,

650   https://doi.org/10.1038/nature11917, 2013.

651   Lü, C. & Tian, H.: Spatial and temporal patterns of nitrogen deposition in China: Synthesis of

652   observational data. J. Geophys. Res., 112, D22S05, https://doi.org/10.1029/2006JD007990,

653   2007.

654   Lu, M., X. Zhou, Q. Yang, H. Li, Y. Luo, C. Fang, J. Chen, X. Yang, and B. Li: Responses of ecosystem

655   carbon cycle to experimental warming: a meta-analysis. Ecology, 94, 726-738,

656   https://doi.org/10.1890/12-0279.1, 2013.

657   Luo, R., J. Fan, W. Wang, J. Luo, Y. Kuzyakov, J.-S. He, H. Chu, and W. Ding: Nitrogen and phosphorus

658   enrichment accelerates soil organic carbon loss in alpine grassland on the Qinghai-Tibetan

659   Plateau. Sci. Total Environ., 650: 303-312, https://doi.org/10.1016/j.scitotenv.2018.09.038,

660   2019.

661   Macias-Fauria, M., B. C. Forbes, P. Zetterberg, and T. Kumpula: Eurasian Arctic greening reveals

662   teleconnections and the potential for structurally novel ecosystems. Nat. Clim. Change, 2, 613-

663   618, https://doi.org/10.1038/nclimate1558, 2012.

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

665    tundra reduced by long-term nutrient fertilization. Nature, 431, 440-443,

666    https://doi.org/10.1038/nature02887, 2004.

667  Mao, J., J. Yao, L. Wang, and W. Liu: Easily prepared high-quantum-yield CdS quantum dots in water

668    using hyperbranched polyethylenimine as modifier. J. Colloid Interf. Sci., 319, 353-356,

669    https://doi.org/10.1016/j.jcis.2007.10.027, 2008.

670  Mathers, N. J., Z. Xu, S. J. Berners-Price, M. S. Perera, and P. G. Saffigna: Hydrofluoric acid pre-

671    treatment for improving ^{13}C CPMAS NMR spectral quality of forest soils in south-east

672    Queensland, Australia. Soil Res., 40, 665-674, https://doi.org/10.1071/SR01073, 2002.

673  Melillo, J. M., S. D. Frey, K. M. Deangelis, W. J. Werner, M. J. Bernard, F. P. Bowles, G. Pold, M. A.

674    Knorr, and A. S. Grandy: Long-term pattern and magnitude of soil carbon feedback to the

675    climate system in a warming world. Science, 358, 101-105,

676    https://doi.org/10.1126/science.aan2874, 2017.

677  Melillo, J. M., P. Steudler, J. D. Aber, K. Newkirk, H. Lux, F. Bowles, C. Catricala, A. Magill, T. Ahrens,

678    and S. Morrisseau: Soil warming and carbon-cycle feedbacks to the climate system. Science,

679    298, 2173-2176, https://doi.org/10.1126/science.1074153, 2002.

680  Meng, J., Li, X.-A: Effects of carbonate on the structure and properties of loess and the corresponding

681    mechanism: an experimental study of the Malan loess, Xi'an area, China. Bull. Eng. Geol.

682    Environ. **78** (7), 4965–4976, https://doi.org/10.1007/s10064-018-01457-z, 2019.

683  Metcalfe, D. B.: Microbial change in warming soils. Science, 358, 41-42,

684    https://doi.org/10.1126/science.aap7325, 2017.

685  Micks, P., Aber, J. D., Boone, R. D., Davidson, E. A.: Short-term soil respiration and nitrogen

686    immobilization response to nitrogen applications in control and nitrogen-enriched temperate

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

688   Mo, J., W. Zhang, W. Zhu, P. Gundersen, Y. Fang, D. Li, and H. Wang: Nitrogen addition reduces soil

689   respiration in a mature tropical forest in southern China. Global Change Biol., 14, 403-412,

690   https://doi.org/10.1111/j.1365-2486.2007.01503.x, 2008.

691   Moscatelli, M. C., A. Lagomarsino, P. De Angelis, and S. Grego: Short-and medium-term contrasting

692   effects of nitrogen fertilization on C and N cycling in a poplar plantation soil. Forest Ecol.

693   Manag., 255, 447-454, https://doi.org/10.1016/j.foreco.2007.09.012, 2008.

694   Nie, M., E. Pendall, C. Bell, C. K. Gasch, S. Raut, S. Tamang, and M. D. Wallenstein: Positive climate

695   feedbacks of soil microbial communities in a semi-arid grassland. Ecol. Lett., 16, 234-241,

696   https://doi.org/10.1111/ele.12034, 2013.

697   Niemeyer, J., Chen, Y., Bollag, J.-M: Characterization of humic acids, composts, and peat by diffuse

698   reflectance Fourier-transform infrared spectroscopy. Soil Sci. Soc. Am., 56, 135–140,

699   https://doi.org/10.2136/sssaj1992.03615995005600010021x, 1992.

700   Ofiti, N. O. E., C. U. Zosso, J. L. Soong, E. F. Solly, M. S. Torn, G. L. B. Wiesenberg, and M. W. I.

701   Schmidt: Warming promotes loss of subsoil carbon through accelerated degradation of plant-

702   derived   organic   matter.   Soil   Biol.   Biochem.,   156,   108185,

703   https://doi.org/10.1016/j.soilbio.2021.108185, 2021.

704   Olk, D. C., G. Brunetti, and N. Senesi: Decrease in Humification of Organic Matter with Intensified

705   Lowland Rice Cropping A Wet Chemical and Spectroscopic Investigation. Soil Sci. Soc. Am.

706   J., 64, 1337-1347, https://doi.org/10.2136/sssaj2000.6441337x, 2000.

707   Paustian, K., J. Lehmann, S. Ogle, D. Reay, G. P. Robertson, and P. Smith: Climate-smart soils. Nature,

708   532, 49-57, https://doi.org/10.1038/nature17174, 2016.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

724   3751, https://doi.org/10.1111/gcb.15101, 2020.

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

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

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

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

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

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

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

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

733   39, https://doi.org/10.1111/j.1365-2486.2006.01263.x, 2007.

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

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

736   40, https://doi.org/10.1016/j.soilbio.2016.12.026, 2017.

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

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

739   114065, https://doi.org/10.1016/j.geoderma.2019.114065, 2020.

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

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

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

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

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

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

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

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

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

749   2389.1997.tb00552.x, 1997.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

794   1987.

795   Vaughn, L. J., and M. S. Torn: ^{14}C evidence that millennial and fast-cycling soil carbon are equally

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

797   0468-y, 2019.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

820   Xiao, J., S. Dong, Z. Zhao, Y. Han, S. Li, H. Shen, and C. Ding: Stabilization of soil organic carbon in

821   the alpine meadow is dependent on the nitrogen deposition level on the Qinghai-Tibetan Plateau.

822   Ecol. Eng., 170, 106348, https://doi.org/10.1016/j.ecoleng.2021.106348, 2021.

823   Xue, K., M. M Yuan, Z. J Shi, Y. Qin, Y. Deng, L. Cheng, L. Wu, Z. He, J. D. Van Nostrand, and R.

824   Bracho: Tundra soil carbon is vulnerable to rapid microbial decomposition under climate

825   warming. Nat. Clim. Change, 6, 595-600, https://doi.org/10.1038/nclimate2940, 2016.

826   Yang, Y., J. Fang, Y. Tang, C. Ji, C. Zheng, J. He, and B. Zhu: Storage, patterns and controls of soil

827   organic carbon in the Tibetan grasslands. Global Change Biol., 14, 1592-1599,

828   https://doi.org/10.1111/j.1365-2486.2008.01591.x, 2008.

829   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,

830   and K. Goulding: Stabilization of atmospheric nitrogen deposition in China over the past decade.

831   Nat. Geosci., 12, 424-429, https://doi.org/10.1038/s41561-019-0352-4, 2019.

832   Zamanian, K., Zarebanadkouki, M., Kuzyakov, Y: Nitrogen fertilization raises CO_2 efflux from inorganic

833   carbon: a global assessment. Glob. Change Biol. 24 (7), 2810–2817,

834   https://doi.org/10.1111/gcb.14148, 2018.

835   Zak, D. R., Z. B. Freedman, R. A. Upchurch, M. Steffens, and I. Kögel-Knabner: Anthropogenic N

836   deposition increases soil organic matter accumulation without altering its biochemical

837   composition. Global Change Biol., 23, 933-944, https://doi.org/10.1111/gcb.13480, 2017.

838   Zhang, H. R., and G. Fu: Responses of plant, soil bacterial and fungal communities to grazing vary with

839   pasture seasons and grassland types, northern Tibet. Land Degrad. Dev., 32: 1821-1832,

840   https://doi.org/10.1002/ldr.3835, 2020.

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

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

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

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

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

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

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

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

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

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

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

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

853   20, 2332-2343, https://doi.org/10.1111/gcb.12490, 2014.

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

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

856   231-242, https://doi.org/10.1111/j.1365-2389.2009.01223.x, 2010.

857

Figure and table legends

Figure 1. Elevation map of the studied sites (a, the pentagram refers to the sampling point), photo of the alpine meadow (b) and the diagram of the warming treatment (open-top chamber) (c).

Figure 2. N and warming-induced changes in the soil properties (mean ± SE, n = 6). Control (white bar) and warmed plots (black bar) at four different levels of simulated N deposition. N0, N1, N2, and N3 indicate N-enrichments of 0, 5, 10, and 15 g N m^{-2} year^{-1}, respectively. Parameters are: Soil pH (a); AGB, aboveground biomass (b); EnC, C-degrading enzymes (c); C/N, ratio of soil C concentration to N concentration (d); MBC, microbial biomass carbo (e); SOC, soil organic carbon stock (f).

Figure 3. N and warming-induced changes in the relative abundance of different functional groups identifiable by diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy in warmed and control plots (mean ± SE, n = 6). The spectral regions were assigned to aromatic carbonyl/carboxyl C=O groups, aromatic C=C groups, lignin-like residues, and cellulose/phenolic.

Figure 4. Four different SOC chemical structural complexity indexes (mean ± SE, n = 6) from solid-state ^{13}C CPMASNMR spectra of soil samples from different treatments. A/O-A=Alkyl C/O-alkyl C; HB/HI = hydrophobic C/hydrophilic C; Alip/Arom = aliphatic C/aromatic C.

Figure 5. The factors regulating the SOC stock under (a) N enrichment, (b) warming treatment and (c)interactive effects of N and warming. In the structural equation model (SEM) analysis, black arrows represent significant positive pathways, gray arrows

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

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

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

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

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

885   Goodness-of-fit statistics for the model are shown as follows: (a), $\chi^2 = 4.53$, $P = 0.53$,

886   GFI =0.99, RMSEA < 0.001; (b), $\chi^2 = 4.47$, $P = 0.486$, GFI = 0.99, RMSEA < 0.001.

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

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

889

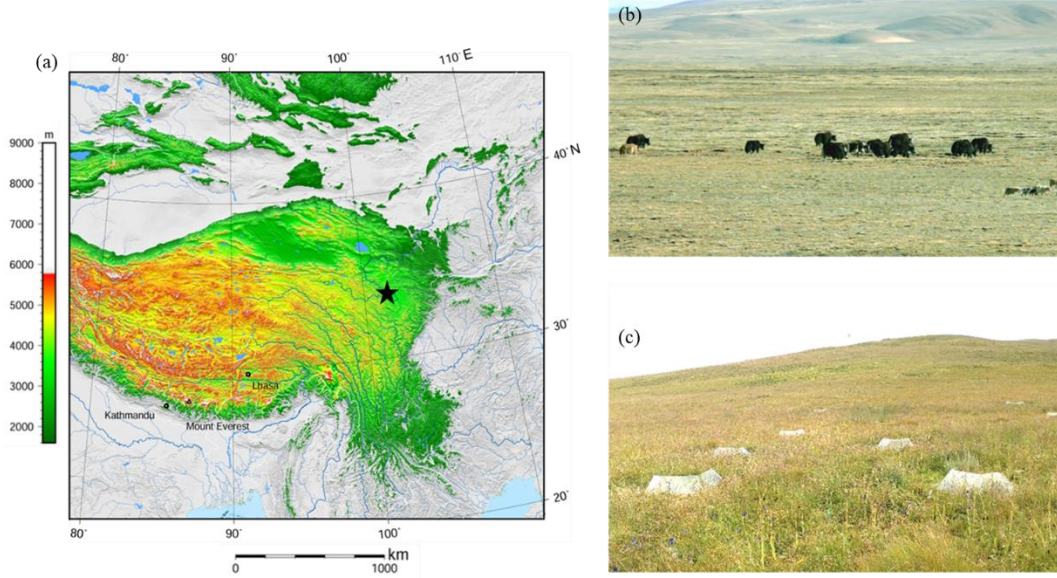
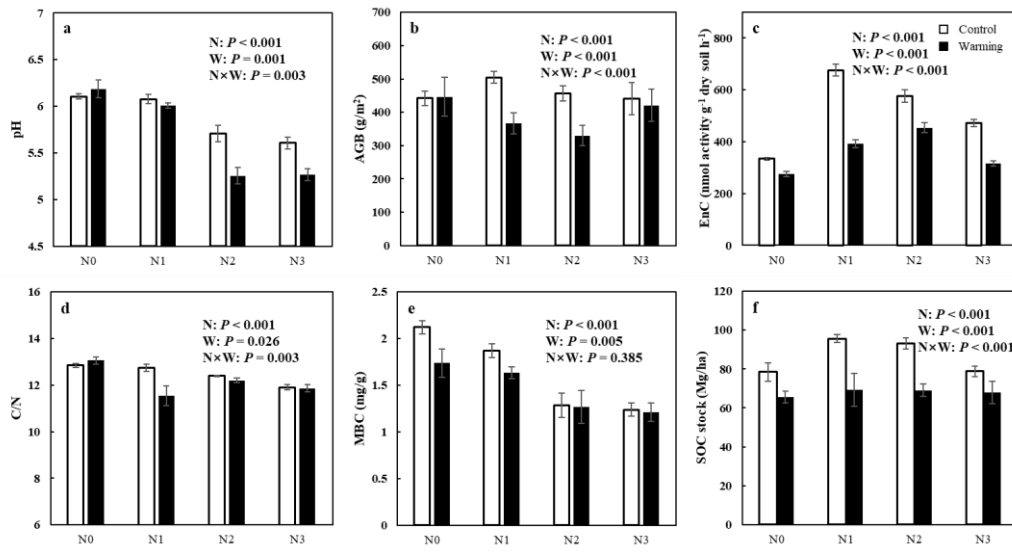
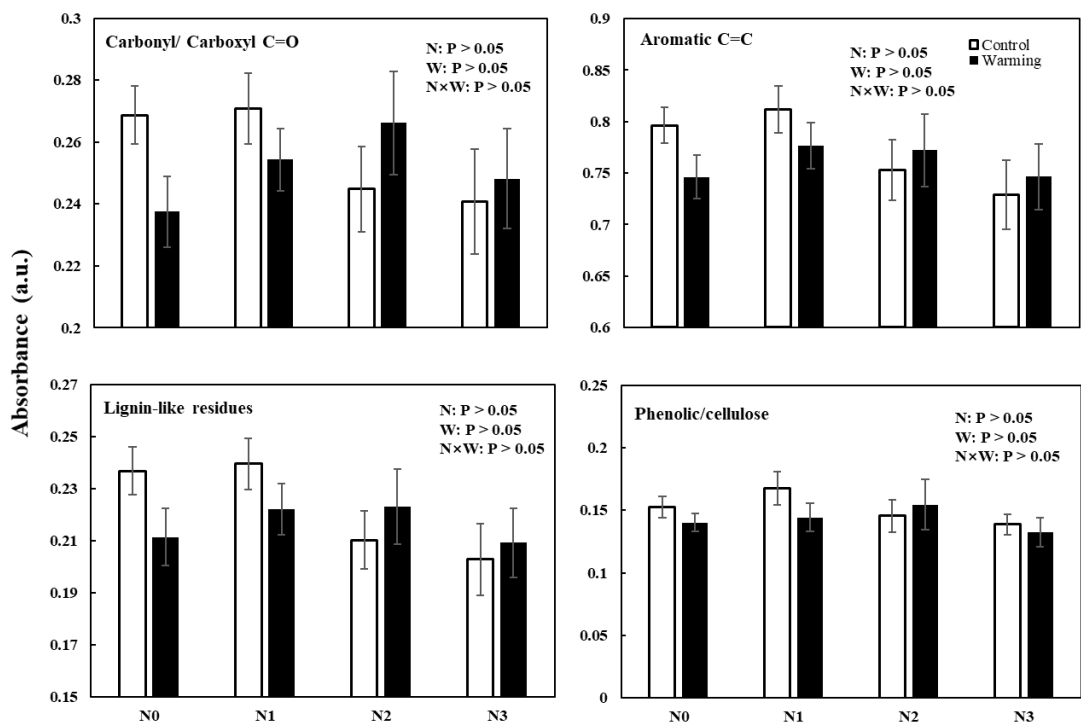



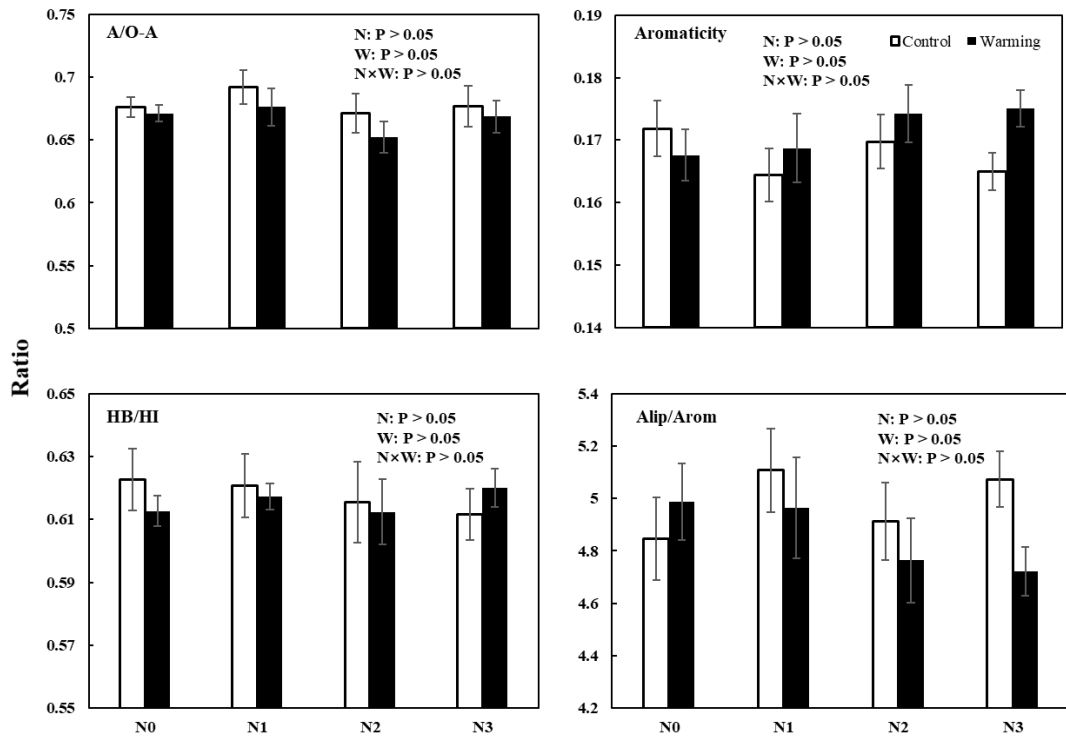
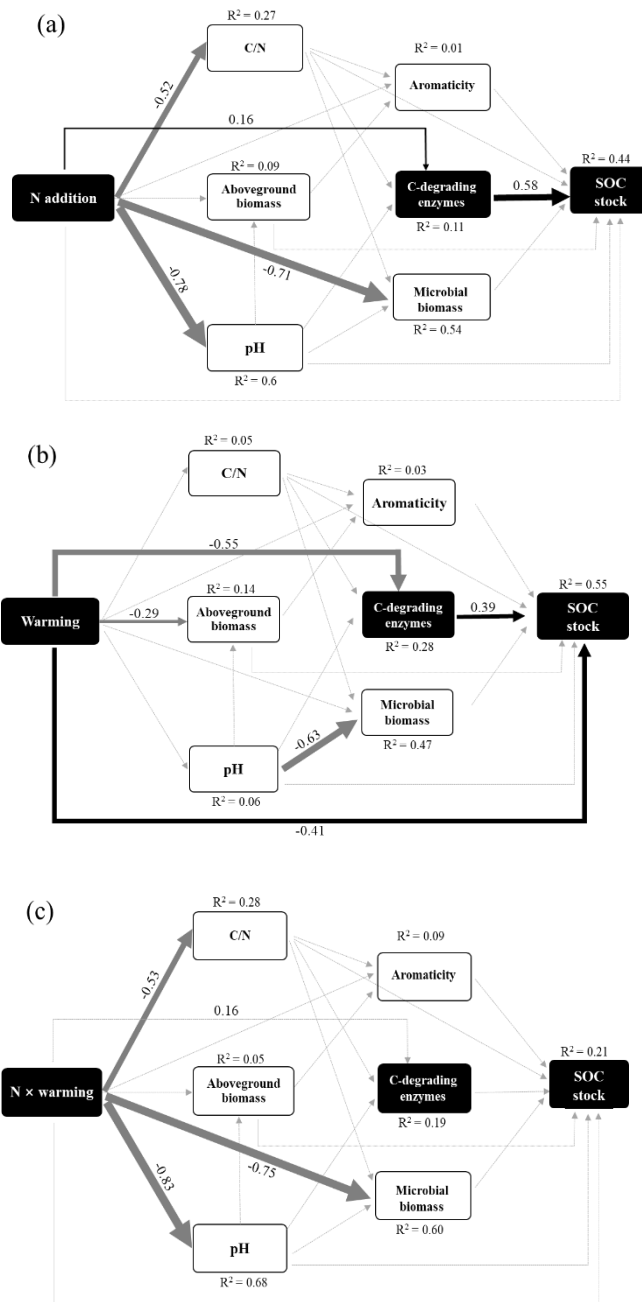
Figure 1

Note: the map (a) was cited from the Wikimedia Commons website (Tibet and surrounding areas above 1600m, created using the Generic Mapping Tools)

895 **Figure 2**

896

897

898 **Figure 3**

899
900

901 **Figure 4**

904 **Figure 5**

905

906

907

908

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
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

^{13}C NMR (%)