



## Effects of warming and grazing on dissolved organic nitrogen in a Tibetan alpine meadow ecosystem

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1 **Warming and grazing directly influence dissolved organic nitrogen in a Tibetan**  
2 **alpine meadow ecosystem**

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31

32 **Abstract** The conversion of insoluble organic nitrogen (N) to dissolved organic N  
33 (DON) is a major constraint to the supply of N to plants, possibly even more so than  
34 the conversion of DON to inorganic N ( $\text{NH}_4^+$  and  $\text{NO}_3^-$ ). The production and fate of  
35 inorganic N as regulated by environmental change are relatively well understood, but  
36 we know comparatively little about how these factors influence DON. We measured  
37 total N, DON and dissolved inorganic nitrogen (DIN) concentrations in the soil  
38 solution and plant N uptake in a factorial warming  $\times$  grazing experiment in a Tibetan  
39 alpine meadow. Results showed that warming significantly decreased DON  
40 concentration by up to 36%. Warming effects on DON were to some extent dependent  
41 on the grazing treatment, and varied with soil depth and sampling date. Grazing  
42 increased soil DON, opposite to the effect of warming. Previous studies have found  
43 warming to increase a range of factors which contribute to the supply of DON in soil;  
44 our results suggest that the observed decrease in DON under warming could be  
45 ascribed to an acceleration of soil DON turnover, greater microbial N immobilization  
46 and enhanced plant N uptake. In conclusion, this study highlights the complex  
47 interaction of land management regime and climate warming in the regulation of  
48 DON cycling in N-limiting environments.

49

50 **Keywords** Alpine meadow • dissolved organic nitrogen • plant N uptake • nitrogen  
51 cycling • progressive N limitation • global warming

52

53 **Introduction**

54 The availability of inorganic nitrogen (N) has traditionally been considered the  
55 primary constraint on vegetation productivity in terrestrial ecosystems (Vitousek and  
56 Howarth, 1991; Elser et al., 2007; LeBauer and Treseder, 2008). However, increasing  
57 studies have shown that the conversion of insoluble organic N to dissolved organic  
58 nitrogen (DON) can also be a major constraint on the supply of N to plants (Jones et  
59 al., 2005; Jan et al., 2009; Jones et al., 2009; Farrell et al., 2011). The production and  
60 fate of inorganic N as regulated by environmental factors, such as temperature and  
61 land use are relatively well understood (Wu et al., 2011; Bai et al., 2013; Ueda et al.,  
62 2013), but relatively few studies have been carried out on how these factors affect  
63 DON, especially in alpine ecosystems.

64 DON concentrations in soil are regulated by a range of factors and represent the  
65 net balance between input and removal processes (Fig 1). Soil DON mainly arises  
66 from plant and microbial turnover and root exudation (Chapman et al., 2001; Jones et  
67 al., 2004; Haynes, 2005; Christou et al., 2006). Similarly, there is increasing evidence  
68 suggesting that plant roots can directly remove DON from soil solution (Jones et al.,  
69 2004; Jones et al., 2005; Xu et al., 2006; Jämtgård et al., 2008; Xu et al., 2011). DON,  
70 however, also represents an important source of C and N for soil microorganisms and  
71 is an important precursor leading to the production of  $\text{NH}_4^+$  and  $\text{NO}_3^-$  in soil (Bardgett  
72 et al., 2003; Butler et al., 2012; Bai et al., 2013) (Fig. 1). Previous studies have shown  
73 inconsistent effects of warming on DON. For example, some experiments show  
74 positive effects as warming accelerates litter decomposition (Kalbitz et al., 2004),  
75 while others demonstrate negative effects in forests, perhaps due to higher  
76 mineralization rates (Huang and Schoenau, 1998; Ueda et al., 2013). Using elevation  
77 as a proxy of climate, results indicate that plants preferentially utilized DON over

78 inorganic N in a cold-temperate forest ecosystem (Averill and Finzi, 2011). Shan et al.  
79 (2014) found that a component of the DON pool (e.g. amino acids) increased with  
80 altitude. Variation in altitude, however, led to marked changes not only in temperature,  
81 but also factors such as moisture and vegetation composition which makes  
82 interpretation of the results difficult. To date, there have no direct, *in situ*  
83 measurements of DON dynamics under elevated temperature.

84 DON includes two functional pools: high molecular weight DON (e.g.  
85 polyphenol-bound protein-N that is recalcitrant and prone to precipitation) and low  
86 molecular weight DON which is highly bioavailable (e.g. amino acids, peptides).  
87 Current evidence suggests that with the exception of inorganic N, plant roots or soil  
88 microorganism only possess the capacity to directly take up and assimilate low  
89 molecular weight DON (Yu et al., 2002; Jones et al., 2004). Further, direct uptake of  
90 soil amino acids by plants has been demonstrated in a range of ecosystems including  
91 cold forest (Persson et al., 2003), arctic tundra (Kielland, 1994; Nordin et al., 2004),  
92 temperate grassland (Weigelt et al., 2005), and Tibet alpine meadow ecosystems (Xu  
93 et al., 2006; Xu et al., 2011).

94 Grazing has been shown to decrease soil DON through the stimulation of net N  
95 mineralization and nitrification (Groffmann et al., 1993; Frank et al., 2000; Le Roux  
96 et al., 2003; Hu et al., 2010). For low productivity ecosystems, however, the opposite  
97 effects of grazing have been observed, showing a decrease in net N mineralization  
98 (Stark et al., 2000; Harrison et al., 2004; Holst et al., 2007). In alpine meadows,  
99 previous results have shown that grazing may stimulate the production of DON as it  
100 increased above- and below-ground plant biomass production (Hu et al., 2010; Wang  
101 et al., 2012) and increased rates of litter decomposition (Luo et al., 2010), while  
102 warming did not affect net N mineralization rates (Wang et al., 2012). We

103 hypothesize that concentrations of DON will increase under grazing in alpine  
104 meadows.

105 Tibetan alpine meadows are unique ecosystems which are particularly sensitive  
106 to global climate change; the average surface temperature in Tibet is expected to  
107 increase 2°C more than the global average by 2050 (Wang and French, 1994;  
108 Thompson et al., 2000; Giorgi et al., 2001). Grazing is the main land use for alpine  
109 meadows, and it is expected that grazing pressure will substantially increase in the  
110 near future due to the rise in human population within the region (Wiener et al., 2003;  
111 Yao et al., 2006). Here, we conducted a field experiment to test the effects of warming  
112 and grazing on soil DON dynamics in Tibetan alpine meadows. First, we tested the  
113 hypothesis that warming decreases soil DON. This could result from greater demand  
114 of DON for plant biomass production (Xu et al., 2006; Wang et al., 2012). Second, we  
115 tested the hypothesis that grazing will increase soil DON as we previously found that  
116 warming increased above- and below-ground plant biomass production (Hu et al.,  
117 2010; Wang et al., 2012) and increased rates of litter decomposition (Luo et al., 2010),  
118 while warming did not affect net N mineralization rates (Wang et al., 2012).

119

## 120 **Materials and methods**

### 121 **Experimental site**

122 The experimental site is located at the Haibei Alpine Meadow Ecosystem Research  
123 Station (37°37'N, 101°12'E), a facility run by the Northwest Institute of Plateau  
124 Biology, Chinese Academy of Sciences. The station lies in the northeastern part of the

125 Qinghai-Tibet plateau in a large valley surrounded by the Qilian Mountains. The mean  
126 elevation of the valley bottom is 3200 m and experiences a typical plateau continental  
127 climate dominated by the southeast monsoons from May to September and  
128 high-pressure systems from Siberia in winter. The climate is characterized by a cold  
129 winter and short, cool summer. The mean annual air temperature is  $-1.7^{\circ}\text{C}$ . The  
130 maximum monthly mean air temperature is  $10^{\circ}\text{C}$  in July and the minimum is  $-15^{\circ}\text{C}$  in  
131 January. Mean temperature and rainfall from 1<sup>st</sup> May to the 20<sup>th</sup> September in 2006  
132 and 2007 (growing season and data collection period) were  $8.4$  and  $8.5^{\circ}\text{C}$ , and 449  
133 and 398 mm, respectively (Luo et al., 2010). The plant community at the experimental  
134 site is dominated by perennial graminoids such as *Kobresia humilis*, *Festuca ovina*,  
135 *Elymus nutans*, *Poa pratensis*, and *Carex scabrirostris* (Wang et al., 2012). The  
136 canopy height of the vegetation in August was about 15–20 cm (Luo et al., 2009).  
137 More than 95% of belowground plant biomass can be found in the upper 20 cm and  
138 fine root biomass is higher at 10-20 cm soil depth than at 0-10 cm depth (Wu et al.,  
139 2011).

140

#### 141 **Controlled warming-grazing experiment**

142 In May 2006, we set up a warming  $\times$  grazing experiment with four replicates for each  
143 treatment combination, i.e., no warming no grazing (NWNG), no warming with  
144 grazing (NWG), warming with no grazing (WNG), and warming with grazing (WG).

145 In total, 16 circular plots (3 m diameter) were used in a complete randomized block  
146 design. For the warming treatments we used an infrared heating system, hereafter



147 called free-air temperature enhancement (FATE) system, as described by Kimball et al.  
148 (2008). In summer (April-October), the set point differences between the heated and  
149 control plots were 1.2°C during daytime and 1.7°C at night, which falls within the  
150 range of the predicted temperature increase for this century for this region (1.5–5°C;  
151 Wang et al., 2012). In winter, the power output of the heaters were manually set at  
152 1500 W per plot.

153 One adult Tibetan domestic sheep (*Ovis aries*) was fenced in the grazing plots on  
154 the morning of 17<sup>th</sup> August 2006 for two hours. Similarly, two adult Tibetan sheep  
155 were fenced for one hour in the grazing plots in the mornings of 12<sup>th</sup> July and 3<sup>rd</sup>  
156 August in 2007. The height of the vegetation was measured at 50 points within the  
157 plots before and after grazing, and the sheep were removed from the grazing plots  
158 when the canopy height was reduced to approximately half of the initial height, which  
159 generally corresponded to a moderate stocking rate in the region (Luo et al., 2009;  
160 Wang et al., 2012).

161

## 162 **Soil temperature and soil moisture**

163 Soil temperature was measured automatically using type-K thermocouples (Campbell  
164 Scientific, Logan, UT, USA) at depths of 5, 10 and 20 cm. All the thermocouples were  
165 connected to a CR1000 datalogger. Soil temperature was measured every minute, and  
166 15 min averages were stored. Soil moisture was manually measured at depths of 10,  
167 20, 30 and 40 cm at 08:00 h, 14:00 h, and 20:00 h daily. All data were collected from  
168 May 26<sup>th</sup> to September 20<sup>th</sup> 2006 and from May 1<sup>st</sup> to September 20<sup>th</sup> in 2007 (Luo et

169 al., 2009).

170

171 **Litter, aboveground and belowground plant biomass, N concentration, and**  
172 **calculated plant N uptake**

173 In August of 2006 and 2007, litter and above- and below-ground plant biomass were  
174 collected from two 10 ×10 cm quadrats in each of the plots. Plant samples were dried  
175 at 80°C for 48 h after which their dry weight was measured. Subsequently,  
176 sub-samples of roots and shoots were ground and used to determine concentrations of  
177 N using a Kjeldahl digestion method with an Alpkem autoanalyzer (Kjektec  
178 System1026 Distilling Unit, Sweden). Above- and below-ground plant N uptake was  
179 calculated multiplying biomass by the N concentration in the respective plant tissue  
180 (Finzi et al., 2007).

181

182 **Soil pore water sampling and analysis**

183 Soil pore water was collected at 2-4 week intervals during two consecutive growing  
184 seasons (10 and 24 June, 10 and 24 July, 16 August in 2006; and 27 May, 10 and 24  
185 July, 24 August in 2007) within 24 h after rainfall using porous-cup ceramic  
186 zero-tension samplers, which were made by the Institute of Geographical Sciences  
187 and Natural Resources Research of the Chinese Academy of Sciences in Beijing.  
188 These samplers were placed at soil depths of 10, 20, 30 and 40 cm in each plot on 24  
189 May in 2006. Soil solution samples were collected from each plot with a vacuum  
190 pump, placed in amber bottles, and stored in a refrigerator at 4°C until further analysis.

191 All samples were filtered through 0.7  $\mu\text{m}$  pore diameter membranes prior to analysis  
192 (Glass Microfibre Filters, GF/F, Whatman, Schleicher and Schuell, England). Total  
193 dissolved N (TDN), dissolved organic C (DOC) and DON, concentrations in the soil  
194 solution were measured using a Shimadzu 5000 TOC/TN analyzer (Kyoto, Japan).  
195 Ammonium-N in the soil solutions was determined colorimetrically by the  
196 salicylate-nitroprusside method of Mulvaney (1996) on a plate reader (Scientific  
197 International, New Delhi, India). Nitrate-N was determined colorimetrically using the  
198 N-1-naphthylethylenediamine method of Miranda et al. (2001) using the same plate  
199 reader. DON was calculated as the difference between the TDN reading and the  
200 amount of dissolved inorganic N (DIN;  $\text{NH}_4^+ + \text{NO}_3^-$ ) present.

201

## 202 **Statistical analysis**

203 Treatment effects on soil TDN, DON concentration, and  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N  
204 concentration were tested using repeated-measures analyses of variance (ANOVA),  
205 with warming, grazing and their interaction as the main factors (between-subject  
206 factors) and with sample date and soil depth as within-subject factors.  
207 Multi-comparisons of least standard difference (LSD) were conducted for all  
208 measured variables within each sampling date and each soil depth using a one-way  
209 ANOVA. Because all plots were free from grazing until 17 August 2006, the data  
210 before that date were analyzed separately. For all ANOVAs, the assumption of  
211 normality was checked with Kolmogorov-Smirnov tests and the assumption of  
212 homogeneity of variances was checked using Levene's tests. If the assumptions were

213 not met, data were log-transformed prior to analysis. Statistical analyses were  
214 performed using SPSS, version 15.0 (SPSS Inc., Chicago, IL, USA).

215 Regression analyses were used to test the relationships between DON  
216 concentration across 0-40 cm soil depth and at individual soil depths (0-10 cm, 10-20  
217 cm, 20-30 cm and 30-40 cm) and the corresponding soil temperature, soil moisture,  
218 soil pH, soil  $\text{NH}_4^+$  and  $\text{NO}_3^-$  concentration, soil total dissolved C concentration and  
219 DOC concentration. Regressions were run separately for 2006 and for 2007 as well as  
220 across the 2006-2007 period. Regressions were run across treatments, but also within  
221 individual treatments. Simple correlation analyses were also performed between  
222 above- or below-ground plant N uptake and the corresponding seasonal mean soil  
223 DON and soil DIN ( $\text{NH}_4^+$ -N and soil  $\text{NO}_3^-$ -N) concentrations. To test the relative  
224 importance of biotic and abiotic factors in determining DON concentration, we ran  
225 stepwise multiple regressions between mean seasonal DON concentration at different  
226 soil depths and various biotic factors (i.e., above- and belowground plant biomass,  
227 above- and below-ground plant N uptake, dead standing plant biomass, and  
228 concentrations of chemical components of dead standing biomass (C content, N  
229 content, cellulose content, hemicellulose content, lignin content and lignin-N content)  
230 and abiotic factors (i.e., soil chemical factors such as, total C, DOC, pH,  $\text{NH}_4^+$ -N and  
231  $\text{NO}_3^-$ -N and soil physical factors such as, temperature and moisture).

232

## 233 **Results**

### 234 **Effects of warming and grazing on soil moisture and temperature**

235 Both warming and grazing did not significantly affect soil moisture content at any of  
236 the 5 measured soil depths in either 2006 or 2007 (Luo et al., 2009). Warming  
237 increased soil temperature in the 0-10 cm soil layer both before and after grazing  
238 (Table S1; Supplementary on-line information). In addition, sheep grazing with or  
239 without external warming also resulted in a significant rise in soil temperature (Table  
240 S1).

241

#### 242 **Effects of warming and grazing on dissolved nitrogen components and** 243 **DOC/DON**

244 Warming significantly decreased total dissolved soil N (TDN) in the 0-40 cm soil  
245 layer by approximately 6% and 17% before and after the grazing treatments started in  
246 2006, respectively (Table 1; Fig. 2), however, there was no effect of warming on TDN  
247 in 2007 (Table 1). For TDN, there was a significant warming  $\times$  soil depth interaction  
248 before grazing started in 2006; warming decreased TDN by 16%, 18% and 7% in the  
249 0-10 cm, 10-20 cm and 20-30 cm soil layers, respectively (Fig. 2).

250 DON constituted a large component of the soluble N in soil, accounting for  
251 approximately 80-90% of the TDN. Warming significantly decreased DON  
252 concentrations before and after the grazing treatments in 2006 by 16% and 10% in the  
253 10-20 and 20-30 cm soil depths respectively, and in 2007 by 36% in the 10-20 cm soil  
254 layer in 2007 (Table 1). After the grazing treatment started, there was a significant  
255 warming  $\times$  grazing  $\times$  soil depth  $\times$  sampling date interaction in 2006 and in 2007  
256 (Table 1), indicating that the effects of warming and grazing on DON were dependent

257 on soil depth and sampling date (Fig. 2, Fig. 3, see also on-line supplementary  
258 information).

259  $\text{NH}_4^+$ -N and  $\text{NO}_3^-$ -N constituted rather small components of the total pool of  
260 soluble soil N, accounting for approximately 10-15% or <10% of the concentration of  
261 TDN, respectively. There was a significant warming  $\times$  date interaction on  $\text{NH}_4^+$ -N  
262 concentrations before and after the grazing treatment started in 2006 (Table 1), with  
263 both positive and negative effects seen depending on season and year (Fig. 2).  
264 Warming decreased  $\text{NO}_3^-$ -N concentration by >50% after the grazing treatments  
265 started in 2006 and in 2007 (Table 1) (Fig. 2). However, effects of warming and  
266 grazing on  $\text{NO}_3^-$ -N concentration were strongly dependent on soil depth and sampling  
267 date.

268 Warming significantly increased the DOC-to-DON ratio in the 0-10 and 10-20  
269 cm soil layers but induced a decrease in the 30-40 cm soil layer prior to the onset of  
270 grazing (see on-line supplementary information Table S2). After grazing, warming a  
271 significant increase in the DOC-to-DON ratio was only seen in the 10-20 cm soil  
272 layer (see on-line supplementary information Table S3).

273

#### 274 **Effects of warming and grazing on litter quality, plant biomass and N uptake**

275 In both years, warming increased the amount of dead standing plant biomass, whereas  
276 grazing significantly decreased the amount of dead standing plant biomass. Warming  
277 and grazing also significantly increased the N concentration in dead standing biomass  
278 (see on-line supplementary information Table S4).

279 Warming significantly increased below-ground plant biomass in 2006 ( $F= 20.54$ ;  
280  $P < 0.001$ ); in the warmed plots, root biomass was on average 25% higher than in the

281 control plots in 2006. Grazing and warming  $\times$  grazing did not significantly affect  
282 below-ground plant biomass in 2006 ( $F = 0.95, 2.60; P = 0.33, 0.11$ , respectively).  
283 There was a significant warming  $\times$  grazing interaction effect on below-ground plant  
284 biomass in 2007 ( $F = 11.31; P < 0.001$ ). Warming significantly increased  
285 above-ground plant biomass production in 2006 and 2007 and there was a weak  
286 warming  $\times$  grazing effect on plant above-ground biomass in 2007; grazing did not  
287 affect plant above-ground biomass in 2006, but significantly reduced plant biomass in  
288 2007 (Wang et al., 2012).

289 Warming significantly increased N uptake in above-ground plant biomass by  
290 approximately 18% in 2006 and 24% in 2007. Warming also increased N uptake in  
291 below-ground plant biomass (upper 20 cm soil depth), up to 25% in 2006 and 35% in  
292 2007 (Table 2; Fig. 4). Grazing did not affect plant above- and below-ground N  
293 uptake in 2006, but grazing significantly decreased N uptake in above-ground plant  
294 biomass by up to 20% and below-ground plant N uptake (upper 20 cm soil depth) by  
295 up to 40% in 2007 (Table 2; Fig. 4). There was a warming  $\times$  grazing effect on  
296 below-ground plant N uptake at 0-10 cm soil depth in 2007 (Table 2).

297

### 298 **Relationships between DON concentration and abiotic and biotic variables**

299 There was a significant positive correlation between DON concentration at 10-20 cm  
300 soil depth and soil temperature in 2006 (Table 3). There were negative correlations  
301 between DON and soil moisture,  $\text{NH}_4^+\text{-N}$ , and pH in 2006 and 2007 at 0-40 cm soil  
302 depth, and between daily DON and  $\text{NO}_3^-\text{-N}$  only for the no warming and no grazing  
303 treatments in 2007 (Table 3). Stepwise regressions showed that the mean seasonal  
304 DON concentration at different soil depths was significantly affected by biotic factors.

305 For example, at 10-20 cm soil depth, a negative correlation was found between DON  
306 and below-ground plant N uptake and plant N uptake explained approximately 42% of  
307 the variation in DON ( $F = 9.97$ ,  $P = 0.007$ ). In the 30-40 cm soil layer, a positive  
308 correlation was found between DON and plant N uptake, which explained  
309 approximately 36% of the variation in DON ( $F = 8.04$ ,  $P = 0.013$ ).

310 At 0-20 cm soil depth in 2006 and 2007, above- and below-ground plant N uptake  
311 was negatively correlated with mean seasonal DON concentrations. No significant  
312 correlations were found between above- and below-ground plant N uptake and  
313 dissolved inorganic N (including  $\text{NH}_4^+$ -N,  $\text{NO}_3^-$ -N) (Fig. 5).

314

## 315 **Discussion**

316 Dissolved organic nitrogen (DON) represents a significant pool of soluble N in many  
317 ecosystems and plays an important role in the N cycling of terrestrial ecosystems  
318 (Jones et al., 2004; Bai et al., 2013; Ueda et al., 2013). Our results showed that DON  
319 accounted for more than 80% of the total dissolved soil N (TDN) in Tibetan alpine  
320 meadows which is consistent with the hypothesis that DON is the quantitatively  
321 dominant pool of high elevation, N-limiting ecosystems (Schimel and Bennett, 2004;  
322 Christou et al., 2005; Kranabetter et al., 2007; Näsholm et al., 2009). For instance,  
323 Farrell et al. (2011) noted that DON is relatively more abundant than DIN in higher  
324 altitude N-limiting grasslands than in productive lowland grasslands. Next we  
325 consider our findings in more detail, in the context of soil DON concentrations and  
326 their dynamics under warming and grazing.

327 Our results supported our hypothesis that warming decreased DON, particularly at



328 10-20 cm soil depths. Among the potential sources of DON, many studies have  
329 argued that DON in soil mainly originates from the decomposition of plant litter  
330 (Kalbitz et al., 2000; Chapman et al., 2001; Haynes 2005) or root exudates (Jones et  
331 al., 1994; Haynes 2005; Jones et al., 2005; Jones et al., 2008; Strickland et al., 2012)  
332 (Fig.1). Our observations at the site have shown that warming increased plant above-  
333 and below-ground biomass, improved the quality of leaf litter, and accelerated litter  
334 decomposition (Luo et al., 2010). This suggests that warming may enhance the  
335 production rates of DON in soil. However, our results showed that warming  
336 significantly decreased DON, particularly at 10-20 cm soil depths (where typically  
337 most of the fine roots were found; Kuzyakov and Xu 2013; Wu et al., 2011). In  
338 general, there are three dominant fates of soil DON in soil solution (Fig. 1): Firstly,  
339 DON can be sorbed to the solid phase, however, this is not likely to be affected by  
340 warming. Secondly, DON can be immobilized by the soil microbial community to  
341 support their nitrogen and/or carbon requirements (Jones et al., 2004, Jones et al.,  
342 2005) and thirdly it can be both converted to  $\text{NH}_4^+$  by the action of both intra- and  
343 extra-cellular enzymes (Bardgett et al., 2003; Jones et al., 2004; Bai et al., 2013).  
344 Warming significantly increased soil microbial biomass-C and -N in this experiment  
345 in 2009 (Rui et al., 2010) suggesting that greater rates of DON immobilization and  
346 mineralization. This is supported by previous studies showing that experimental  
347 warming enhances the decomposition of soil organic N and mineralization via  
348 increased microbial activity (e.g., Bardgett et al., 2008; Luo et al., 2010). Our results  
349 showed that warming decreased DON in both 2006 and 2007, with the difference

350 evident early in the growing season and at the end of the growing season (Fig. 2). This  
351 decrease in DON is temporally consistent with increased soil temperatures, microbial  
352 activity and soil respiration (by ca. 10%) occurring at the same time (Lin et al., 2011)  
353 supporting the view that loss of DON was due to accelerated DON decomposition.  
354 However, this hypothesis is not consistent with Wang et al. (2012) who found that  
355 warming did not significantly affect soil net N mineralization at the same site. It  
356 should be noted, however, that the measurement of net N mineralization ignored plant  
357 DIN uptake and it is possible that warming increased gross N mineralization.

358 Plants roots and their associated mycorrhizas can directly take up low molecular  
359 weight DON to support plant growth (Jones et al., 2004; Jones et al., 2005; Xu et al.,  
360 2006; Jämtgård et al., 2008; Xu et al., 2011). This DON loss pathway within our  
361 experiment is potentially supported by a significant negative correlation between plant  
362 N uptake and soil DON (Fig. 5). Moreover, plant N uptake explained approximately  
363 42% of variation in DON concentration at 10-20 cm soil depth. In 2007, warming  
364 decreased DON at 10-20 cm soil depth by  $8.34 \text{ g m}^{-2}$ , while plant N uptake increased  
365 by  $11.72 \text{ g m}^{-2}$ . However, Xu et al. (2006) suggests that plants take up a maximum of  
366 30% of N as organic N (Xu et al., 2006, 2011) indicating that soil organic N would  
367 only decrease by  $3.51 \text{ g m}^{-2} \text{ y}^{-1}$ . Further, experiments using dual-labeled  
368  $^{13}\text{C}$ - $^{15}\text{N}$ -glycine showed that the warming decreased plant capture of amino acid-N  
369 from soil (Ma et al., 2015 under review). The results of Xu et al. (2006, 2011) and Ma  
370 et al. (2015), however, must be placed against the huge uncertainty inherent in  
371 measuring and interpreting the uptake of  $^{15}\text{N}$ -DON by plants (Jones et al., 2005). In

372 addition, recent studies in N-limiting environments suggest that plants compete at a  
373 higher level in the N breakdown pathway by taking up small peptides from soil,  
374 thereby by-passing the need to take up amino acids,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  (Hill et al.,  
375 2011a,b, 2012). Further work is therefore required to determine the chemical nature of  
376 DON in these soils and to determine their relative availability to both plants and  
377 microorganisms.

378 DON could also potentially leave this ecosystem via leaching (Hu et al., 2010). In  
379 our experiment we conclude that leaching is probably of minor importance due to the  
380 lack of observed differences in soil moisture content and the inherently low rates of  
381 water mobility (Zhu et al., 2011).

382 Previous studies at the site showed that warming increased soil temperature by 0.5  
383 to 1.5 °C across all soil depths both in 2006 and in 2007, while grazing only increased  
384 soil temperature in 2007. In this study, we found that there was only a weak positive  
385 correlation between soil temperature with DON ( $r^2 = 0.08$ ) and a weak negative  
386 correlation between soil moisture and DON concentration ( $r^2 = 0.05$ ). This indicates  
387 that abiotic factors probably only play a minor role in directly modulating DON  
388 concentrations in Tibet alpine meadow ecosystems. The poor relationships of these  
389 abiotic variables with soil DON also highlights the potential influence of biotic factors  
390 in regulating soil DON concentrations (Lü et al., 2014).

391 Our results confirmed our hypothesis that grazing increased soil DON,  
392 opposite to the effect of warming. Overall, grazing increased below-ground plant  
393 biomass production (Hu et al., 2010; Wang et al., 2012) and increased rates of litter

394 decomposition (Luo et al., 2010), both of which are known to positively influence  
395 rates of DON production. Grazing could also influence soil DON by: (1) increasing  
396 soil temperature, changing soil moisture status (Asner et al., 2004; Christou et al.,  
397 2005) or by inducing alterations in soil structure and hydrological flow pathways (via  
398 sheep trampling). This can produce a complex range of both negative and positive  
399 feedbacks on rates of DON production and consumption (Holst et al., 2007; Wu et al.,  
400 2011; Houst et al., 2007; Kauffman et al., 2004); (2) Grazing can reduce rates of  
401 litterfall and can decrease litter quality (Luo et al., 2011), which can decrease N both  
402 in the plant (Fig. 4) and microbial N pools (Rui et al., 2011). Our results showed that  
403 grazing decreased above- and below-ground plant total N uptake by about 25% which  
404 could have further contributed to the loss of DON through plant uptake; and (3) Our  
405 results show that grazing strongly increases soil  $\text{NO}_3^-$  concentrations. This result is  
406 consistent with the founding of Rui et al. (2011) in 2009 at the same site and those of  
407 Wu et al. (2011) in the Inner Mongolia steppes. The increase in  $\text{NO}_3^-$  concentrations  
408 under grazing could be explained by the addition of sheep excreta especially at the  
409 soil surface (Fig. 3).

410

## 411 **Conclusions**

412 Our results showed that warming decreased soil DON concentrations and that  
413 conversely, grazing increased soil DON concentrations. We suggest that a  
414 warming-induced reduction in DON can be ascribed to accelerated rates of plant and  
415 microbial uptake of DON rather than changes in DON production rate or leaching

416 losses. Further work is therefore required to (1) characterize and quantify the chemical  
417 nature of DON, (2) determine the biological fate of DON under warming and grazing  
418 treatment, and (3) develop modeling tools to enable prediction of anthropogenically  
419 mediated changes in environment on DON pool or fluxes.

420

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427

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