- Soil  $\delta^{15}$ N is a better indicator of ecosystem nitrogen cycling than 1
- plant  $\delta^{15}$ N: A global meta-analysis 2
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12	<b>Abstract.</b> The nitrogen-15 ( <sup>15</sup> N) natural abundance composition ( $\delta^{15}$ N) in soils or
13	plants is a useful tool to indicate the openness of ecosystem N cycling. This study was
14	aimed to evaluate the influence of the experimental warming on soil and plant $\delta^{15}N$ .
15	We applied a global meta-analysis method to synthesize 79 and 76 paired
16	observations of soil and plant $\delta^{15}N$ from 20 published studies, respectively. Results
17	showed that the mean effect sizes of the soil and plant $\delta^{15}N$ under experimental
18	warming were -0.524 (95% CI: -0.987 to -0.162) and 0.189 (95% CI: -0.210 to 0.569),
19	respectively. This indicated that soil $\delta^{15}$ N had negative response to warming at the
20	global scale, where warming had no significant effect on plant $\delta^{15}N$ . Experimental
21	warming significantly ( $p < 0.05$ ) decreased soil $\delta^{15}$ N in Alkali and medium-textured
22	soils, in grassland/meadow, under air warming, for 4-10 yr warming period and for an
23	increase of > 3 °C in temperature, whereas it significantly ( $p < 0.05$ ) increased soil
24	$\delta^{15}N$ in neutral and fine-textured soils and for an increase of 1.5-3 $^{o}C$ in temperature.
25	Plant $\delta^{15}$ N significantly ( $p < 0.05$ ) increased with increasing temperature in neutral
26	and fine-textured soils and significantly ( $p < 0.05$ ) decreased in alkali soil. Latitude
27	did not affect the warming effects on both soil and plant $\delta^{15}N$ . However, the warming
28	effect on soil $\delta^{15}N$ was positively controlled by the mean annual temperature, which is
29	related to the fact that the higher temperature can strengthen the activity of soil
30	microbes. The effect of warming on plant $\delta^{15}N$ had weaker relationships with
31	environmental variables compared with that on soil $\delta^{15}N$ . This implied that soil $\delta^{15}N$
32	was more effective than plant $\delta^{15}N$ in indicating the openness of global ecosystem N
33	cycling.

#### 34 **1 Introduction**

Nitrogen (N) is one of the most important nutrient elements for plant growth and the 35 key limiting factors for vegetation productivity (McLay et al., 2001; Zhu et al., 2018; 36 37 Lu et al., 2020). On the one hand, if the available N in the soil is insufficient, it will damage and weaken the ecosystem service function, including the supply of primary 38 material products, water conservation, climate regulation, etc. (Averill and Waring, 39 2018). On the other hand, if the available N in the soil is over supplied, it will also 40 damage the structure and function of the ecosystem, resulting in a series of 41 42 environmental problems such as soil acidification and imbalance of ecosystem 43 nutrient (Schrijver et al., 2008). The intermediate products of the N cycling processes, such as nitrate nitrogen  $(NO_3^- - N)$ , nitrous oxide  $(N_2O)$  and nitric oxide (NO), may 44 also cause eco-environmental pollution such as eutrophication of water body and 45 aggravation of climate-related issues (Liao et al., 2019). Therefore, it is of great 46 significance to reveal the openness of the ecosystem N cycle process for 47 understanding the plant N fixation and long-term trend of N cycling and protecting 48 the eco-environment (Wang et al., 2014; Wu et al., 2019). Openness is a measure of 49 both N inputs and outputs relative to internal cycling and determines both the 50 potential rate of N accumulation in the ecosystem and the potential for N losses 51 following a disturbance (Rastetter et al., 2021). 52

53 The <sup>15</sup>N natural abundance composition ( $\delta^{15}$ N) in soils or plants (leaves, shoots, 54 fine roots and litter) is often used to indicate the openness of ecosystem N cycling 55 (Robinson, 2001). This is because the lighter isotope of <sup>14</sup>N is always preferentially

56	lost from the ecosystem. Thus, the isotopic fractionation effect results in gradual $^{15}$ N
57	enrichment in the ecosystem (Aranibar et al., 2004). The larger the $\delta^{15}N$ value, the
58	higher degree of openness of N cycling. In addition, soil $\delta^{15}N$ also appears to reflect
59	the degree of decomposition of the organic matter, showing that $\delta^{15}N$ increases with
60	processing (Craine et al., 2015). A large number of studies have confirmed that
61	climate was the main factor regulating the soil and plant $\delta^{15}N$ (Craine et al., 2015;
62	Soper et al., 2015). Previous studies have demonstrated that precipitation had a
63	negative effect on soil and plant $\delta^{15}N$ from in-situ evidences to cross-sites syntheses
64	(Swap et al., 2004; Soper et al., 2015). However, the influence of temperature on soil
65	and plant $\delta^{15}N$ remains controversial. Some studies have shown that soil and plant
66	$\delta^{15}$ N increased with temperature (Amundson et al., 2003; Craine et al., 2015), while
67	others have indicated that $\delta^{15}$ N decreased with temperature (Cheng et al., 2009; Sheng
68	et al., 2014) or that they were not correlated (Yang et al., 2013). The various studies
69	suggested that the responses of soil and plant $\delta^{15}N$ to warming were very complex and
70	not well understood. In addition to climate factor, soil and plant $\delta^{15}N$ are affected by a
71	variety of other environmental factors, such as vegetation type, topography, soil
72	properties and management practices (Gurmesa et al., 2017; Wang et al., 2019).
73	However, we know little about the influences of environmental factors on the
74	warming effect on ecosystem N cycling, in terms of soil and plant $\delta^{15}$ N.
75	Soil and air warming experiments have often been conducted to study the effect
76	of warming on the ecosystem N cycling at site scale (Schindlbacher et al., 2009). At

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present, the effect of experimental warming on soil and plant  $\delta^{15}N$  has not been

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studied on a global scale. The objectives of this study were to: (i) detect the effect of 78 experimental warming on the soil and plant  $\delta^{15}$ N based on a global meta-analysis of 79 20 studies; and (ii) identify the main factors influencing the warming effect on the soil 80 and plant  $\delta^{15}$ N. In addition, previous studies (e.g., Liu and Wang, 2009; Wang et al., 81 2014) have found that the correlation between soil  $\delta^{15}N$  and environmental factors 82 was stronger than that for plant, which may be due to the fact that soil samples 83 represented a long-term average for a given location, while plant samples were 84 affected by the microenvironment or the short-term environmental fluctuations. 85 Therefore, we specifically hypothesized that soil  $\delta^{15}N$  is a better indicator of 86 ecosystem N cycling than plant  $\delta^{15}$ N. 87

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## 88 2 Materials and methods

## 89 2.1 Source of data and selection criteria

Peer-reviewed journal articles and dissertations related to soil and plant δ<sup>15</sup>N under
experimental warming were searched using Web of Science and China National
Knowledge Infrastructure (CNKI, <u>http://www.cnki.net</u>) until March 31, 2020 (Tab. 1).
The keywords used for the literature search were related to: "nitrogen isotope
composition", "experimental warming" and "ecosystems nitrogen cycling".

Our criteria were as follows: at least one of the target variables was contained, including soils (different fractions, e.g., sand, silt, clay, aggregate and bulk soil) and plants (leaves, shoots, roots and litters)  $\delta^{15}N$ ; studies with climate gradients (space-time substitution) were excluded and only field warming experimental studies were included; only data from control and warming treatments were applied for multifactor experiments; means, standard deviations (SD) (or standard errors (SE)) and sample sizes were directly provided or could be calculated from the studies; if one article contained soil or plant  $\delta^{15}$ N in multiple years, only the latest results were applied since the observations should be independent in the meta-analysis (Hedges et al., 1999).

105 2.2 Data extraction and statistical analysis

In total, 20 published papers were selected from 54 published papers. The locations of 106 warming experiments were presented and their site information is listed in Tab. 1. For 107 108 each study, the means, the statistical variation (SE or SD) and the sample size values for treatment and control groups were extracted for each response variable ( $\delta^{15}$ N). In 109 addition to  $\delta^{15}N$ , the latitude, longitude, altitude, soil pH, organic matter content, 110 vegetation type, mean annual precipitation (MAP) and mean annual temperature 111 (MAT) were also extracted if they were provided (Tab. 1). All data were extracted 112 from tables or digitized from graphs with the software GetData v2.2.4 113 (http://www.getdata-graph-digitizer.com). A total of 79 and 76 paired observations for 114 soil and plant  $\delta^{15}$ N were obtained, respectively. 115

The METAWIN 2.1 software (Sinauer Associates Inc., Sunderland, MA, USA) (Rosenberg et al., 2000) was used to perform meta-analysis in this study. The Hedges' *d* value was used as the effect size (Hedges et al., 1999). The absolute *d* value indicated the magnitude of the treatment impact. Positive or negative *d* values represented an increase or decrease effect of the treatment, respectively. Zero meant no difference between treatment and control groups. Resampling tests were

incorporated into our meta-analysis using the bootstrap method (999 random 122 replicates). The mean effect size (calculated from 999 iterations) and 95% bootstrap 123 124 confidence intervals (CI) were then generated. If the 95% CI values of d did not overlap zero, the effects of experimental warming on  $\delta^{15}N$  were considered significant 125 at p < 0.05. We used a random effects model to test whether warming had a significant 126 effect on  $\delta^{15}$ N. To examine whether experimental conditions alter the response 127 direction and magnitude of soil and plant  $\delta^{15}$ N, observations were further divided into 128 subgroups according to the soil acidity-alkalinity (acid (pH < 6.5), neutral (6.5 < pH < 129 130 7.5), and alkali (pH > 7.5), vegetation types (forest/shrub, moss/lichen, and 131 grassland/meadow), warming treatments (soil warming, air warming, and both soil and air warming), soil texture (fine-, medium-, and coarse-textured soil), length of 132 warming (< 4 yr, 4-10 yr, and > 10 yr), and increase in temperature (<  $1.5 \degree C$ ,  $1.5-3 \degree C$ , 133 and > 3 °C). A random effects model with a grouping variable was used to compare 134 responses among different subgroups. Linear regression analyses were applied to 135 assess the relationships between the Hedges' d values and environmental factors (i.e., 136 latitude, altitude, MAT and MAP). 137

# 138 **3 Results**

Across all sites, the mean effect sizes of the soil and plant  $\delta^{15}$ N under experimental warming were -0.524 (95% CI: -0.987 to -0.162) and 0.189 (95% CI: -0.210 to 0.569), respectively (Fig. 1). Experimental warming significantly (p < 0.05) decreased soil  $\delta^{15}$ N in Alkali (mean effect size = -2.484; 95% CI: -2.931 to -2.060) and medium-textured (mean effect size = -0.676; 95% CI: -1.153 to -0.249) soils, in

grassland/meadow (mean effect size = -0.609; 95% CI: -1.076 to -0.190), under air 144 warming (mean effect size = -0.652; 95% CI: -1.081 to -0.273), for 4-10 yr warming 145 period (mean effect size = -0.652; 95% CI: -1.081 to -0.273) and for an increase of > 146 147 3 °C in temperature (mean effect size = -0.652; 95% CI: -1.081 to -0.273). However, it significantly (p < 0.05) increased soil  $\delta^{15}$ N in neutral (mean effect size = 0.359; 95%) 148 CI: 0.078 to 0.620) and fine-texture soils (mean effect size = 2.394; 95% CI: 1.770 to 149 3.735), and for an increase of 1.5-3  $^{\circ}$ C in temperature (mean effect size = 0.409; 95%) 150 CI: 0.070 to 0.707) (Fig. 2). Experimental warming did not significantly (p > 0.05)151 change soil  $\delta^{15}$ N under other experimental conditions. 152

In addition, experimental warming significantly (p < 0.05) increased plant  $\delta^{15}$ N in neutral (mean effect size = 3.157; 95% CI: 1.529 to 6.967) and fine-textured soils (mean effect size = 1.202; 95% CI: 1.042 to 1.360), whereas it significantly (p < 0.05) decreased plant  $\delta^{15}$ N in alkali soil (mean effect size = -1.930; 95% CI: -2.325 to -1.573) (Fig. 2). Experimental warming did not significantly (p > 0.05) change plant  $\delta^{15}$ N under other experimental conditions.

For soil and plant  $\delta^{15}$ N, their responses to experimental warming did not correlate well with latitude (p = 0.268 and p = 0.160, respectively) (Fig. 3ab). However, the Hedges' *d* values of soil  $\delta^{15}$ N decreased significantly with altitude (p < 0.001) (Fig. 3c) and increased significantly with MAT (p < 0.001) and MAP (p < 0.001) (Fig. 3eg). In addition, the Hedges' *d* values of plant  $\delta^{15}$ N were also found to increase significantly with MAP (p < 0.001) (Fig. 3h). However, the responses of plant  $\delta^{15}$ N to experimental warming did not correlate well with altitude (p = 0.109) and MAT (p = 0.002) (Fig. 166 3df).

## 167 **4 Discussion**

A significant decreasing trend in soil  $\delta^{15}N$  and no significant trend in plant  $\delta^{15}N$  were 168 169 found in this study. This is somewhat inconsistent with previous findings. Chang et al. (2017) observed that soil and plant  $\delta^{15}N$  values decreased under warming in the 170 Tibetan permafrost. However, Zhang et al. (2019) found that the warming treatment 171 significantly increased soil and plant  $\delta^{15}$ N in a subtropical forest. The various studies 172 suggest that soil and plant  $\delta^{15}$ N are controlled by interactive effects of N fixation and 173 mineralization. At the global scale,  $\delta^{15}N$  of N input (~ 0) is generally lower than that 174 of soil, so greater N fixation or higher N input (deposition and fertilization) under 175 warming can result in a lower soil  $\delta^{15}N$  (Sorensen and Michelsen, 2011; Rousk and 176 Michelsen, 2017; Wang et al., 2018). 177

Soil pH has an important influence on nitrification, denitrification and N2O 178 emissions from soils (Kyveryga et al., 2004). The results in this study showed that 179 when the soil was alkaline, the mean effect sizes of soil and plant  $\delta^{15}$ N under warming 180 were negative, while when the soil was neutral, they were positive (Fig. 2ab). 181 Compared with alkaline condition, the near neutral conditions are more suitable for 182 the biological activities of heterotrophic denitrifying bacteria (Simek and Cooper, 183 2002). Therefore, the denitrification activity is usually higher under neutral conditions, 184 resulting in an enrichment of soil and plant N pools with <sup>15</sup>N (Kyveryga et al., 2004). 185 Vegetation type had limited effects on  $\delta^{15}N$  under warming, except for soil  $\delta^{15}N$  in 186 grassland/meadow (Fig. 2cd). This may be related to the differences in altitude, MAP 187

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188	and MAT among three vegetation types (Tab. 1). The type of warming treatment was
189	found to have a substantial effect on soil $\delta^{15}N$ , showing that the mean effect size of
190	soil $\delta^{15}$ N under air warming was negative and less than that under soil warming (Fig.
191	2ef). Salmon et al. (2016) have found that soil warming can increase N availability by
192	stimulating mineralization of organic matter in the warmed active layer. In addition,
193	air warming directly impacts aboveground temperatures and has an indirect effect on
194	soil $\delta^{15}N$ (Pardo et al., 2006). From Fig. 2gh, the finer the soil texture, the more
195	significant the positive effect of warming on soil and plant $\delta^{15}N$ . The possible reason
196	is that the finer the soil texture, the stronger the adsorption of various ions on the soil
197	and the smaller the leaching loss of the soil, resulting in greater residual amount of
198	<sup>15</sup> N in the soil (Webster et al., 1986). In addition, the longer warming period and the
199	greater increase in temperature resulted in the more negative effect of warming on soil
200	$\delta^{15}N$ (Fig. 2ik). Chang et al. (2017) deduced that N fixation was greater under
201	warming and consequently resulted in a lower soil $\delta^{15}$ N.

In the study of Mayor et al. (2015), soil and plant  $\delta^{15}N$  were significantly (p < 1202 0.001) and negatively correlated with latitude at the global scale. However, the 203 Hedges' d values of soil and plant  $\delta^{15}$ N had weak correlations with latitude in this 204 study (Fig. 3). The warming effect on soil  $\delta^{15}N$  was significantly (p < 0.001) 205 influenced by altitude, MAT and MAP. Among these, the strongest correlation was 206 observed for MAT. It is possible that soil  $\delta^{15}$ N increased with increasing MAT when 207 the MAT exceeded a certain threshold (e.g., 9.8 °C as proposed by Craine et al. 208 (2015)). In this case, the increase in MAT can enhance the positive effect of 209

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210	experimental warming on soil $\delta^{15}N$ . In addition, the MAT can also affect ecosystem N
211	cycle by influencing soil texture. Craine et al. (2015) reported that hot sites had
212	greater clay concentrations than cold sites. As depicted in Fig. 2g, the finer the texture
213	of the soil, the more significant the effect of experimental warming on soil $\delta^{15}$ N. High
214	d values of soil $\delta^{15}$ N corresponded to MAT of about 20 °C, which was the most
215	suitable temperature for nitrification and denitrification. However, warming had a
216	substantial negative impact on soil $\delta^{15}N$ when MAT decreased to around -5 °C.
217	Recently, Rousk et al. (2018) also found that the increase of temperature in the Arctic
218	promoted the biological N fixation, which can decrease the soil $\delta^{15}$ N. The decrease of
219	d values of soil $\delta^{15}$ N with increasing altitude and decreasing MAP in this study might
220	be caused by the positive response of $d$ values to MAT.
221	The relationships between the $d$ values and environmental variables for plant
222	$\delta^{15}$ N were weaker than those for soil $\delta^{15}$ N (Fig. 3). The possible reason is that several

experimental warming on soil  $\delta^{15}$ N. In addition, the MAT can also affect access M

 $\delta^{15}$ N were weaker than those for soil  $\delta^{15}$ N (Fig. 3). The possible reason is that several 222 other factors (e.g., plant N concentrations and species richness) might co-regulate 223 plant  $\delta^{15}$ N (Wu et al., 2019). This is consistent with the study of Craine et al. (2009), 224 who found different inflection points in soil and plant  $\delta^{15}$ N relationships with MAT. In 225 addition, plants are generally depleted in <sup>15</sup>N relative to soils. Above results implied 226 that soil  $\delta^{15}N$  was more efficient in indicating the openness of ecosystem N cycling 227 than plant  $\delta^{15}$ N at the global scale. Although the present study provided a global 228 meta-analysis of the responses of  $\delta^{15}$ N to experimental warming, the magnitude of 229 these responses might be uncertain. For example, a small number of observations 230 were obtained in moss/lichen under soil warming and both soil and air warming 231

批注 [f11]: Response to the comment 10 from Topical Editor. treatments, which would affect the results of meta-analysis. Future research should take more experimental data into account in order to better investigate the warming effects on  $\delta^{15}$ N.

235 6 Conclusions

Our global meta-analysis indicated a significant decreasing trend in soil  $\delta^{15}N$  and no 236 significant trend in plant  $\delta^{15}$ N under experimental warming. Latitude did not affect the 237 warming effects on  $\delta^{15}$ N. However, the warming effect on  $\delta^{15}$ N was related to soil 238 acidity-alkalinity, texture, vegetation type, warming treatment and period, increase in 239 temperature, altitude, MAT and MAP. The effect of warming on soil  $\delta^{15}$ N was better 240 correlated with environmental variables compared with that on plant  $\delta^{15}N$ . Our 241 findings should be useful for understanding the underlying mechanisms of the 242 response of ecosystem N cycling to global warming. 243

Data availability. The data that support the findings of this study are available fromthe corresponding author upon request.

Author contributions. KL and QZ designed this study, KL and XL performed the meta-analysis, KL and QZ obtained funding, and KL and XL wrote the paper with contributions from QZ.

249 **Competing interests.** The authors declare that they have no conflict of interest.

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259	References
260	Aerts, R., Callaghan, T. V., Dorrepaal, E., van Logtestijn, R. S. P., and Cornelissen, J.
261	H. C.: Blackwell Publishing Ltd Seasonal climate manipulations result in
262	species-specific changes in leaf nutrient levels and isotopic composition in a
263	sub-arctic bog. Funct. Ecol., 23, 680–688,
264	https://doi.org/10.1111/j.1365-2435.2009.01566.x, 2009.
265	Amundson, R., Austin, A. T., Schuur, E. A. G., Yoo, K., Matzek, V., Kendall, C.,
266	Uebersax, A., Brenner, D., and Baisden, W.T.: Global patterns of the isotopic
267	composition of soil and plant nitrogen. Global Biogeochem. Cy., 17, 1031,
268	https://doi.org/10.1029/2002GB001903, 2003.
269	Anadon-Rosell, A., Ninot, J. M., Palacio, S., Grau, O., Nogués, S., Navarro, E.,
270	Carmen Sancho, M., and Carrillo, E.: Four years of experimental warming do not
271	modify the interaction between subalpine shrub species. Oecologia, 183, 1167-
272	1181, https://doi.org/10.1007/S00442-017-3830-7, 2017.
273	Aranibar, J. N., Otter, L., Macko, S. A., Feral, C. J. W., Epstein, H. E., Dowty, P. R.,
274	Eckardt, F., Shugart, H. H., and Swap, R. J.: Nitrogen cycling in the soil-plant

system along a precipitation gradient in the Kalahari sands. Global Change Biol., 275

- 276 10, 359–373, https://doi.org/10.1111/j.1365-2486.2003.00698.x, 2004.
- 277 Averill, C., and Waring, B.: Nitrogen limitation of decomposition and decay: How
- can it occur? Global Change Biol., 4, 1417–1427,
- 279 https://doi.org/10.1111/gcb.13980, 2018.
- 280 Bijoor, N. S., Czimczik, C. I., Pataki, D. E., and Billings, S. A.: Effects of temperature
- and fertilization on nitrogen cycling and community composition of an urban
- 282 lawn. Global Change Biol., 14, 2119–2131,
- 283 https://doi.org/10.1111/j.1365-2486.2008.01617.x, 2008.
- 284 Chang, R., Wang, G., Yang, Y., and Chen, X.: Experimental warming increased soil
- nitrogen sink in the Tibetan permafrost. J. Geophys. Res. Biogeosci., 122, 1870–
  1879, https://doi.org/10.1002/2017JG003827, 2017.
- 287 Cheng, W. X., Chen, Q. S., Xu, Y. Q., Han, X. G., and Li, L. H.: Climate and
- ecosystem <sup>15</sup>N natural abundance along a transect of Inner Mongolian grasslands:
- 289 Contrasting regional patterns and global patterns. Global Biogeochem. Cy., 23,
- 290 GB2005, https://doi.org/10.1029/2008GB003315, 2009.
- 291 Cheng, X., Luo, Y., Xu, X., Sherry, R., and Zhang, Q.: Soil organic matter dynamics

in a North America tallgrass prairie after 9 yr of experimental warming.

293 Biogeosciences, 8, 1487–1498, https://doi.org/10.5194/bg-8-1487-2011, 2011.

- Craine, J. M. et al.: Convergence of soil nitrogen isotopes across global climate
  gradients. Sci. Rep., 5, 8280, https://doi.org/10.1038/srep08280, 2015.
- 296 Dawes, M. A., Schleppi, P., Hättenschwiler, S., Rixen, C., and Hagedorn, F.: Soil
- warming opens the nitrogen cycle at the alpine treeline. Global Change Biol., 23,

- 298 421–434, https://doi.org/10.1111/gcb.13365, 2017.
- 299 Deane-Coe, K. K., Mauritz, M., Celis, G., Salmon, V., Crummer, K. G., Natali, S. M.,
- 300 and Schuur, E. A. G.: Experimental warming alters productivity and isotopic
- 301 signatures of tundra mosses. Ecosystems, 18, 1070–1082,
- 302 https://doi.org/10.1007/s00442-015-3427-y, 2015.
- 303 Gonzalez-Meler, M. A., Silva, L. B. C., Dias-De-Oliveira, E., Flower, C. E., and
- 304 Martinez, C. A.: Experimental air warming of a Stylosanthes capitata, vogel
- 305 dominated tropical pasture affects soil respiration and nitrogen dynamics. Front.
- 306 Plant sci., 8, 46, https://doi.org/10.3389/fpls.2017.00046, 2017.
- 307 Gurmesa, G. A., Lu, X. K., Gundersen, P., Fang, Y. T., Mao, Q. G., Hao, C., and Mo,
- J. M.: Nitrogen input N-15 signatures are reflected in plant N-15 natural
  abundances in subtropical forests in China. Biogeosciences, 14, 2359–2370,
- 310 https://doi.org/10.5194/bg-14-2359-2017, 2017.
- 311 Hedges, L. V., Gurevitch, J., and Curtis, P. S.: The meta-analysis of response ratios in
- experimental ecology. Ecology, 80, 1150–1156,
- 313 https://doi.org/10.1890/0012-9658(1999)080[1150:TMAORR]2.0.CO;2, 1999.
- 314 Hudson, J. M. G., Henry, G. H. R., and Cornwell, W. K.: Taller and larger: shifts in
- 315 Arctic tundra leaf traits after 16 years of experimental warming. Global Change
- Biol., 17, 1013–1021, https://doi.org/10.1111/j.1365-2486.2010.02294.x, 2011.
- 317 Kyveryga, P. M., Blackmer, A. M., Ellsworth, J. W., and Isla, R.: Soil pH effects on
- nitrification of fall-applied anhydrous ammonia. Soil Sci. Soc. Am. J., 68, 545–
- 319 551, https://doi.org/10.2136/sssaj2004.0545, 2004.

320	Liao,	K.,	Lai,	Х.,	Zhou,	Ζ.,	Zeng,	Х.,	Xie,	W.,	Castellano,	М.	J.,	and	Zhu,	Q.:
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- 321 Whether the rock fragment content should be considered when investigating
- nitrogen cycle in stony soils? J. Geophys. Res. Biogeosci., 124, 521-536,
- 323 https://doi.org/10.1029/2018JG004780, 2019.
- Lim, H., Oren, R., Näsholm, T., Strömgren, M., Lundmark, T., Grip, H., and Linder,
- 325 S.: Boreal forest biomass accumulation is not increased by two decades of soil
- 326 warming. Nat. Clim. Change, 9, 49–52,
- 327 <u>https://doi.org/10.1038/s41558-018-0373-9</u>, 2019.
- 328 Liu, W., and Wang, Z.: Nitrogen isotopic composition of plant-soil in the Loess
- 329 Plateau and its responding to environmental change. Chin. Sci. Bull., 54, 272–
- 330 279, <u>https://doi.org/10.1007/s11434-008-0442-y</u>, 2009.
- 331 Lu, Y., Gao, Y., and Yang, T.: A review of mass flux monitoring and estimation
- methods for biogeochemical interface processes in watersheds. J. Geogr. Sci., 30,
- 333 881–907, https://doi.org/10.1007/s11442-020-1760-5, 2020.
- 334 Lv, C., Zhang, Q., Hao, Y., Chen, Y., and Yang, Y.: Influence of short-term warming
- 335 on the composition of stable carbon and nitrogen isotopes in *Cunninghamia*
- 336 lanceolata in subtropical region of China. Forest Res., 31, 27–32,

337 https://doi.org/10.13275/j.cnki.lykxyj.2018.05.004, 2018. (in Chinese)

- Mayor, J., Bahram, M., Henkel, T., Buegger, F., Pritsch, K., and Tedersoo, L.:
  Ectomycorrhizal impacts on plant nitrogen nutrition: emerging isotopic patterns,
  latitudinal variation and hidden mechanisms. Ecol. Lett., 18, 96–107,
- 341 https://doi.org/10.1111/ele.12377, 2015.

- 342 McLay, C., Dragten, R., Sparling, G., and Selvarajah, N.: Predicting groundwater
- 343 nitrate concentrations in a region of mixed agricultural land use, a comparison of
- three approaches. Environ. Pollut., 115, 191–204,
- 345 https://doi.org/10.1016/S0269-7491(01)00111-7, 2001.
- 346 Munir, T. M., Khadka, B., Xu, B., and Strack, M.: Mineral nitrogen and phosphorus
- 347 pools affected by water table lowering and warming in a boreal forested peatland.
- 348 Ecology, 10, e1893, https://doi.org/10.1002/eco.1893, 2017.
- 349 Natali, S. M., Schuur, E. A. G., and Rubin, R. L.: Increased plant productivity in
- Alaskan tundra as a result of experimental warming of soil and permafrost. J.

351 Ecol., 100, 488–498, https://doi.org/10.1111/j.1365-2745.2011.01925.x, 2012.

- Pardo, L. H., et al.: Regional assessment of N saturation using foliar and root  $\delta^{15}$ N. Biogeochemistry, 80, 143–171, https://doi.org/10.1007/s10533-006-9015-9,
- 354 2006.
- 355 Peng, A.: Effects of artificial warming on alpine meadow in the permafrost region of
- 356 Qinghai-Tibet Plateau. Master dissertation, The University of Chinese Academy
- 357 of Sciences, 2017. (in Chinese)
- Rastetter, E. B., Kling, G. W., Shaver, G. R., Crump, B. C., Gough, L., and Griffin, K. 358 L.: Ecosystem recovery from disturbance is constrained by N cycle openness, 359 vegetation-soil N distribution, form of N losses, and the balance between 360 361 vegetation and soil-microbial processes. Ecosystems, 24, 667-685, https://doi.org/10.1007/s10021-020-00542-3, 2021. 362
- 363 Robinson, D.:  $\delta^{15}$ N as an integrator of the nitrogen cycle. Trends Ecol. Evol. (Amst.),

- 364 16, 153–162, https://doi.org/10.1016/S0169-5347(00)02098-X, 2001.
- 365 Rosenberg, M., Adams, D., and Gurevitch, J.: MetaWin: Statistical Software for
- 366 Meta-Analysis. Sinauer Associates, Sunderland, MA, USA, 2000.
- 367 Rousk, K., and Michelsen, A.: Ecosystem nitrogen fixation throughout the snow-free
- 368 period in subarctic tundra: Effects of willow and birch litter addition and
- 369 warming. Global Change Biol., 23, 1552–1563,
- 370 https://doi.org/10.1111/gcb.13418, 2017.
- 371 Rousk, K., Sorensen, P. L., and Michelsen, A.: What drives biological nitrogen
- fixation in high arctic tundra: Moisture or temperature? Ecosphere, 9, e02117,
   https://doi.org/10.1002/ecs2.2117, 2018.
- 374 Rui, Y., Wang, S., Xu, Z., Wang, Y., Chen, C., Zhou, X., Kang, X., Lu, S., Hu, Y.,
- 375 Lin, Q., and Luo, C.: Warming and grazing affect soil labile carbon and nitrogen
- pools differently in an alpine meadow of the Qinghai–Tibet Plateau in China. J.
- 377 Soils Sediments, 11, 903–914, <u>https://doi.org/10.1007/s11368-011-0388-6</u>, 2011.
- 378 Salmon, V. G., Soucy, P., Mauritz, M., Celis, G., Natali, S. M., Mack, M. C., and
- 379 Schuur, E. A. G.: Nitrogen availability increases in a tundra ecosystem during
- five years of experimental permafrost thaw. Global Change Biol., 22, 1927–1941,
- 381 <u>https://doi.org/10.1111/gcb.13204</u>, 2016.
- 382 Schaeffer, S. M., Sharp, E., Schimel, J. A. P., and Welker, J. M.: Soil-plant N
- 383 processes in a High Arctic ecosystem, NW Greenland are altered by long-term
- experimental warming and higher rainfall. Global Change Biol., 19, 3529–3539,
- 385 https://doi.org/10.1111/gcb.12318, 2013.

- 386 Schindlbacher, A., Zechmeister-Boltenstern, S., and Jandl, R.: Carbon losses due to
- 387 soil warming: do autotrophic and heterotrophic soil respiration respond equally?
- 388 Global Change Biol., 15, 901–913,
- 389 https://doi.org/10.1111/j.1365-2486.2008.01757.x, 2009.
- 390 Schnecker, J., Borken, W., Schindlbacher, A., and Wanek, W.: Little effects on soil
- 391 organic matter chemistry of density fractions after seven years of forest soil
- 392 warming. Soil Biol. Biochem., 103, 300–307,
- 393 https://doi.org/10.1016/j.soilbio.2016.09.003, 2016.
- 394 Schrijver, A. D., Verheyen, K., Mertens, J., Staelens, J., Wuyts, K., and Muys, B.:
- Nitrogen saturation and net ecosystem production. Nature, 451, E1,
  https://doi.org/10.1038/nature06578, 2008.
- 397 Sheng, W., Yu, G., Fang, H., Liu, Y., Wang, Q., Chen, Z., and Zhang, L.: Regional
- $^{398}$  patterns of  $^{15}$ N natural abundance in forest ecosystems along a large transect in
- 399 eastern China. Sci. Rep., 4, 4249, <u>https://doi.org/10.1038/srep04249</u>, 2014.
- 400 Simek, M., and Cooper, J. E.: The influence of soil pH on denitrification: progress
- 401 towards the understanding of this interaction over the last 50 years. Eur. J. Soil

402 Sci., 53, 345–354, https://doi.org/10.1046/j.1365-2389.2002.00461.x, 2002.

403 Soper, F. M., Richards, A. E., Siddique, I., Aidar, M. P. M., Cook, G. D., Hutley, L.

- 404 B., Robinson, N., and Schmidt, S.: Natural abundance  $(\delta^{15}N)$  indicates shifts in
- nitrogen relations of woody taxa along a savanna-woodland continental rainfall
- 406 gradient. Oecologia, 178, 297–308, https://doi.org/10.1007/s00442-014-3176-3,
- 407 2015.

- 408 Sorensen, P. L., and Michelsen, A.: Long-term warming and litter addition affects
- 409 nitrogen fixation in a subarctic heath. Global Change Biol., 17, 528–537,
- 410 <u>https://doi.org/10.1111/j.1365-2486.2010.02234.x</u>, 2011.
- 411 Swap, R. J., Aranibar, J. N., Dowty, P. R., Gilhooly, W. P., and Macko, S. A.: Natural
- 412 abundance of  ${}^{13}$ C and  ${}^{15}$ N in C<sub>3</sub> and C<sub>4</sub> vegetation of southern Africa: patterns
- 413 and implications. Global Change Biol., 10, 350–358,
- 414 https://doi.org/10.1111/j.1365-2486.2003.00702.x, 2004.
- 415 Wang, C., Wang, X., Liu, D., Wu, H., Lv, X., Fang, Y., Cheng, W., Luo, W., Jiang, P.,
- 416 Shi, J., Yin, H., Zhou, J., Han, X., and Bai, E.: Aridity threshold in controlling
- 417 ecosystem nitrogen cycling in arid and semi-arid grasslands. Nat. Commun., 5,
- 418 4799, https://doi.org/10.1038/ncomms5799, 2014.
- 419 Wang, Q., Nian, J., Xie, X., Yu, H., Zhang, J., Bai, J., Dong, G., Hu, J., Bai, B., Chen,
- 420 L., Xie, Q., Feng, J., Yang, X., Peng, J., Chen, F., Qian, Q., Li, J., and Zuo, J.:
- 421 Genetic variations in *ARE1* mediate grain field by modulating nitrogen
- 422 utilization in rice. Nat. Commun., 9, 735,
- 423 https://doi.org/10.1038/s41467-017-02781-w, 2018.
- 424 Wang, X., Jiang, Y., Ren, H., Yu, F., and Li, M.: Leaf and soil  $\delta^{15}N$  patterns along
- 425 elevational gradients at both treelines and shrublines in three different climate
- 426 zones. Forests, 10, 557, https://doi.org/10.3390/f10070557, 2019.
- 427 Webster, C.P., Belford, R.K., and Cannell, R.Q.: Crop uptake and leaching losses of
- 428 15N labelled fertilizer nitrogen in relation to waterlogging of clay and sandy
- 429 loam soils. Plant Soil, 92, 89–101, 1986.

430	Wu, J., Song, M., Ma, W., Zhang, X., Shen, Z., Tarolli, P., Wurst, S., Shi, P.,												
431	Ratzmann, G., Feng, Y., Li, M., Wang, X., and Tietjen, B.: Plant and soil's $\delta^{15}N$												
432	are regulated by climate, soil nutrients, and species diversity in alpine grasslands												
433	on the northern Tibetan Plateau. Agr. Ecosyst. Environ., 281, 111-123,												
434	https://doi.org/10.1016/j.agee.2019.05.011, 2019.												
435	Yang, Y. H., Ji, C. J., Robinson, D., Zhu, B., Fang, H. J., Shen, H. H., and Fang, J. Y.:												
436	Vegetation and soil <sup>15</sup> N natural abundance in alpine grasslands on the tibetan												
437	plateau: patterns and implications. Ecosystems, 16, 1013-1024,												
438	https://doi.org/10.1007/s10021-013-9664-1, 2013.												
439	Zhang, Q., Zhou, J., Li, X., Yang, Z., Zheng, Y., Wang, J., Lin, W., Xie, J., Chen, Y.,												

- and Yang, Y.: Are the combined effects of warming and drought on foliar
  C:N:P:K stoichiometry in a subtropical forest greater than their individual effects?
  For. Ecol. Manage., 448, 256–266, https://doi.org/10.1016/j.foreco.2019.06.021,
  2019.
- Zhao, Y., Xu, L., Yao, B., Ma, Z., Zhang, C., Wang, F., and Zhou, H.: Influence of simulated warming to the carbon, nitrogen and their stability isotope-(δ<sup>13</sup>C, δ<sup>15</sup>N)
  contents in alpine meadow plant leaves. Acta Bot. Boreal Occident Sin., 36, 777–783, https://doi.org/10.7606/j.issn.1000-4025.2016.04.0777, 2016. (in Chinese)
- Zhu, Q., Castellano, M. J., and Yang, G. S.: Coupling soil water processes and the
  nitrogen cycle across spatial scales: Potentials, bottlenecks and solutions.
  Earth-Sci. Rev., 187, 248–258, https://doi.org/10.1016/j.earscirev.2018.10.005,

452 2018.

References	Country/Region	Vegetation types	Soil pH	Soil type	OMC <sup>a</sup> (%)	Latitude	Longitude	Altitude (m a.s.l)	MAT <sup>b</sup> (°C)	MAP <sup>c</sup> (mm)
Anadon-Rosell et al. (2017)	Spain	Subalpine shrub	4.49~4.6 3	Mineral soil	13.15~14.04	41.39 N	2.17 E	2250	3	1146.4
Zhang et al. (2019)	China	C. lanceolata seedlings	5.07	Oxisol	-	26.32 N	117.6 E	300	19.1	1670
Lim et al. (2019)	Sweden	Boreal forests	5.92~6.4 4	A thin, podzolic, sandy soil	-	64.12 N	19.45 E	310	2.4	600
Deane-Coe et al. (2015)	USA	Tundra mosses	-	Gelisol	-	63.88 N	149.23 W	700	-2.7~-1	138~228
Bijoor et al. (2008)	USA	Turfgrass lawn	-	Alkaline alo clay	-	33.7 N	117.7 W	30	18.6	352
Chang et al. (2017)	China	Alpine meadow	9.1~9.3	Gelisols	5.5	34.73 N	92.89 E	4750	-5.3	269.7
Gonzalez-Meler et al. (2017)	Brazil	Grasslands	5.0	Dystrophic red latosols	-	21.17 S	47.86 W	578	21.5	1100
Natali et al. (2012)	USA	Shrubs, sedges and mosses	-	Gelisol	-	63.88 N	149.23 W	700	-1	178~250
Munir et al. (2017)	Canada	Shrubs, mosses and trees	-	-	-	55.27 N	112.47 W			
Salmon et al. (2016)	USA	Eriophorum vaginatum	-	Gelisols	-	63.88 N	149.23 W	700	-1.45	200
Rui et al. (2011)	China	Alpine	-	-	-	37.62 N	101.2 °E	3200	-2	500

**Table 1:** Site characteristics from a global meta-analysis of 20 studies.

		meadow								
Aerts et al.		Shrubs,								
(2009)	Sweden	mosses and	-	-	-	68.35 N	18.82 E	340	0.5	303
		trees								
Cheng et al.	USA	Tallgrass	Neutral	Nash-Lucien complex	-	34.98 N	97.52 W		16	911.4
(2011)		prairie	pН	Ĩ						
Dawes et al.	Switzerland	Alpine	-	Sandy Ranker and Podzols	-	46.77 N	9.87 E	2180	9.2	444
(2017)		treeline		·						
Schaeffer et al.		Prostrate					<0 <b>3</b> 1		1.0	200
(2013)	Greenland	dwarf-shrub	-	Turbic cryosols	-	76 N	68 W		4~8	<200
		herb tundra	Naar	A mosaic of shallow						
Schnecker et al.	Austria	Spruce forest	Near neutral	A mosaic of shallow Chromic Cambisols and	8.55~14.96	47.58 N	11.64 E	910	6.9	1506
(2016)		Spruce lorest	pH	Rendzic Leptosols	8.55~14.90	47.JO IN	11.04 E	910	0.9	1500
		Heath,	pm	Reliuzie Leptosois						
Hudson et al.	Canada	willow and	-	_	_	78.88 N	75.78 W		8.6~10.	
(2011)		meadow							4	
		Cunninghami								
Lv et al. (2018)	China	a lanceolata	-	Red soil	2.21	26.32 N	118 E		19.1	1585
~ /		juveniles								
Zhao et al. (2016)		Alpine				27 40 21	101.0 7	2200 2250	1 5	<b>600</b>
	China	meadow	-	Alpine meadow soils	-	37.48 N	101.2 E	3200~3250	-1.7	600
$D_{2} = (2017)$	China	Alpine		A 1		24 72 91	02 90 T	2200 4900	5.02	
Peng (2017)	China	meadow	-	Alpine meadow soils	-	34.73 N	92.89 E	3200~4800	-5.03	267.4~426.3

<sup>a</sup>Soil organic matter content; <sup>b</sup>Mean annual temperature; <sup>c</sup>Mean annual precipitation. If the soil organic carbon content was provided in the literature, soil

organic matter content was determined by multiplying the organic carbon content by a coefficient of 1.724.

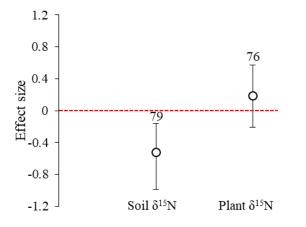
## **List of Figures:**

**Figure 1:** Effect sizes of the experimental warming on soil and plant  $\delta^{15}$ N from a global meta-analysis of 20 studies. The error bars indicate effect sizes and 95% bootstrap confidence intervals (CI). The warming effect was statistically significant if the 95% CI did not bracket zero. The sample size for each variable is shown next to the bar.

**Figure 2:** Factors influencing the effect sizes of the soil and plant  $\delta^{15}$ N under experimental warming from a global meta-analysis of 20 studies, including (a-b) soil acidity-alkalinity, (c-d) vegetation types, (e-f) warming treatments, (g-h) soil texture, (i-j) length of warming and (k-l) increase in temperature. The error bars indicate effect sizes and 95% bootstrap confidence intervals (CI). The warming effect was statistically significant if the 95% CI did not bracket zero. The sample size for each variable is shown next to the bar.

**Figure 3:** Relationships between the Hedges' *d* values of soil and plant  $\delta^{15}$ N with the latitude, altitude, mean annual temperature (MAT) and mean annual precipitation (MAP) under experimental warming.





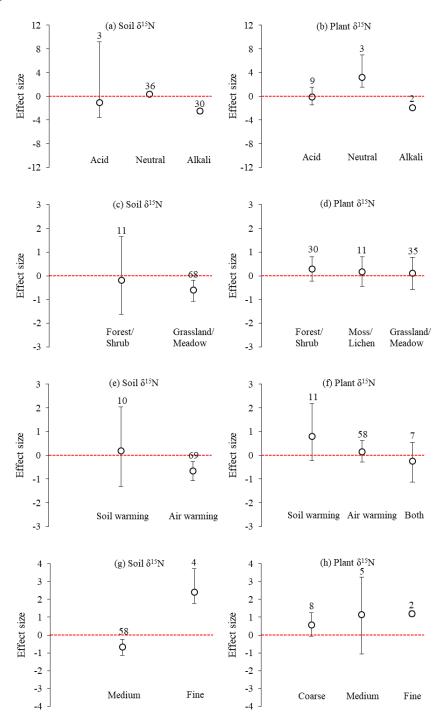


Figure 2

