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Nitrogen isotopic composition of plants and soil in an arid mountainous terrain: south slope versus north slope

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23 **Abstract**

24 Nitrogen cycling is tightly associated with environment. South slope of a given
25 mountain could significantly differ from north slope in environment. Thus, N cycling
26 should also be different between the two slopes. Since leaf $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$ and
27 $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ ($\Delta\delta^{15}\text{N}_{\text{leaf-soil}} = \text{leaf } \delta^{15}\text{N} - \text{soil } \delta^{15}\text{N}$) could reflect the N cycling
28 characteristics, we put forward a hypothesis that leaf $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$ and $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$
29 should differ across the two slopes. However, such a comparative study between two
30 slopes has never been conducted yet. In addition, environmental effects on leaf and
31 soil $\delta^{15}\text{N}$ derived from studies at global scale were often found to be different from
32 that at regional scale. This led to our argument that environmental effects on leaf and
33 soil $\delta^{15}\text{N}$ could depend on local environment. To confirm our hypothesis and
34 argument, we measured leaf and soil $\delta^{15}\text{N}$ on the south and north slopes of Mount
35 Tianshan. Remarkable environment differences between the two slopes provided an
36 ideal opportunity for our test. The study showed that leaf $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$ and
37 $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ on the south slope were greater than that on the north slope although the
38 difference in soil $\delta^{15}\text{N}$ was not significant. The result confirmed our hypothesis and
39 suggested that the south slope has higher soil N transformation rates and soil N
40 availability than the north slope. Besides, this study observed that the significant
41 influential factors of leaf $\delta^{15}\text{N}$ were temperature, precipitation, leaf N, leaf C/N, soil
42 moisture and silt/clay ratio on the north slope, whereas on the south slope only leaf
43 C/N was related to leaf $\delta^{15}\text{N}$. The significant influential factors of soil $\delta^{15}\text{N}$ were
44 temperature, precipitation, soil moisture and silt/clay ratio on the north slope,

45 whereas on the south slope, MAP and soil moisture exerted significant effects.
46 Precipitation exerted contrary effects on soil $\delta^{15}\text{N}$ between the two slopes. Thus, this
47 study supported our argument that the relationships between leaf and soil $\delta^{15}\text{N}$ and
48 environmental factors are localized.

49

50 **1 Introduction**

51 In natural terrestrial ecosystem, nitrogen (N) is not only the most required element,
52 but also is usually a key limiting resource for plants (Vitousek et al., 1997), thus,
53 studying N cycling is of vital importance. The variations of nitrogen isotope ratio
54 ($\delta^{15}\text{N}$) in plants and soil are tightly associated with many biogeochemical processes
55 including N mineralization, ammonia volatilization, nitrification, denitrification
56 (Högberg, 1997; Houlton et al., 2006). Mineralization produces available N,
57 including ammonium and nitrate, which are the substrates for ammonia volatilization,
58 nitrification and denitrification. During these processes, gaseous N loss is more
59 likely to be depleted in ^{15}N , which will cause the remaining N pool and subsequently
60 plants to enrich ^{15}N (Högberg, 1997). Additionally, the difference between leaf $\delta^{15}\text{N}$
61 and soil $\delta^{15}\text{N}$ ($\Delta\delta^{15}\text{N}_{\text{leaf-soil}} = \text{leaf } \delta^{15}\text{N} - \text{soil } \delta^{15}\text{N}$), which is also named enrichment
62 factor (Emmett et al., 1998), was also suggested to be an indicator of ecosystem N
63 cycling (Charles and Garten, 1993; Kahmen et al., 2008; Fang et al., 2011), and it
64 was also reported to be correlated with soil N transformation rates (N mineralization
65 or nitrification rates) (Garten and Van Miegroet, 1994). Thus, nitrogen isotopes have
66 been widely applied in studies of terrestrial ecosystem N cycling (Handley et al.,

67 1999; Evans, 2001; Robinson, 2001; Hobbie and Colpaert, 2003; Houlton et al.,
68 2007).

69 For a given mountain, its south slope may be significantly different from its north
70 slope in climate and environment. It is well known that ecosystem N cycling is
71 associated with climatic and environmental conditions (Amundson et al., 2003;
72 Craine et al., 2009; Yang et al., 2013; Zhou et al., 2016), thus, ecosystem N cycling
73 should vary across south and north slopes. Since leaf $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$ and $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$
74 could reflect and indicate ecosystem N cycling, differences in leaf $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$
75 and $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ were expected to appear between south and north slopes.
76 Comparisons on leaf $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$ and $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ across two slopes of a
77 mountain would provide a good insight into the response of terrestrial ecosystem N
78 cycling to climate and environment. However, to our knowledge, such a comparative
79 study has never been conducted yet.

80 Most of the published works consistently suggested that leaf $\delta^{15}\text{N}$ increased with
81 increasing mean annual temperature (MAT) and decreasing mean annual
82 precipitation (MAP) at large regional or global scales (Austin and Sala, 1999;
83 Amundson et al., 2003; Craine et al., 2009). However, in contrast to the commonly
84 reported patterns, leaf $\delta^{15}\text{N}$ was found to be negatively related to MAT in some
85 Asian regions, e.g., in Inner Mongolian (Cheng et al., 2009) and eastern China
86 (Sheng et al., 2014). Relative to plant $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$ has been little addressed. Some
87 studies demonstrated that soil $\delta^{15}\text{N}$ decreased with increasing MAP and decreasing
88 MAT at the global scale (Amundson et al., 2003; Craine et al., 2015b). However,

89 studies based on local or region scale showed inconsistent results with the global
90 patterns. Cheng et al. (2009) reported that soil $\delta^{15}\text{N}$ increased with decreasing MAT
91 in Inner Mongolian. Sheng et al. (2014) showed that the soil $\delta^{15}\text{N}$ in tropical forest
92 ecosystems were ^{15}N -depleted than in temperate forest ecosystems of eastern China.
93 Yang et al. (2013) found that soil $\delta^{15}\text{N}$ did not vary with either MAT or MAP on the
94 Tibetan Plateau. Wang et al. (2014) revealed a second-order polynomial relationship
95 between soil $\delta^{15}\text{N}$ and aridity index across arid and semi-arid regions. The above
96 inconsistent observations led to our argument that the relationships between
97 environmental factors and leaf $\delta^{15}\text{N}$ or soil $\delta^{15}\text{N}$ would depend on local environment.
98 Comparisons on the effects of climatic and environmental factors on leaf $\delta^{15}\text{N}$ and
99 soil $\delta^{15}\text{N}$ between south and north slopes of a given mountain could test the
100 argument.

101 This study was conducted on the south slope and the north slope of Mount
102 Tianshan. It is an ideal place for testing our hypotheses because its south slope
103 differs greatly from its north slope in climatic and environmental conditions (Deng et
104 al., 2015; Zhang et al., 2016). The first objective of the present study was to confirm
105 our hypothesis that the south slope differs from the north slope in leaf $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$
106 and $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$. The second objective was to test our argument that environmental
107 effects on leaf $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ are localized.

108

109 **2 Materials and methods**

110 **2.1 Study area**

111 Mount Tianshan is one of the largest seven mountains over the world. It has a total
112 length of 2500 km straddling four countries including China, Kazakhstan,
113 Kyrgyzstan and Uzbekistan. In China, Mount Tianshan stretches 1700 km along the
114 east-west direction in the Xinjiang Uygur Autonomous Region and covers about
115 570,000 square kilometers and accounts for one third of the whole area. Mount
116 Tianshan divides Xinjiang into two parts, the south of Tianshan is the Tarim Basin
117 and the north is the Dzungaria Basin.

118 This study was conducted along an elevation transect on the north and south
119 slopes on eastern Mount Tianshan ($42.43^{\circ} - 43.53^{\circ}\text{N}$, $86.23^{\circ} - 87.32^{\circ}\text{E}$) (Fig.1).
120 Mount Tianshan is characterized by an arid mountainous climate; vertical variations
121 in temperature and precipitation are very pronounced, temperature decreases and
122 precipitation increases with altitude on both slopes. The north slope differs
123 significantly from the south slope both in climate and vegetation. On the north slope,
124 the annual mean temperature (MAT) ranges from -6.40°C to 3.90°C with the
125 average temperature of -1.85°C , and the annual mean precipitation (MAP) ranges
126 from 314 mm to 472 mm with the average precipitation of 402 mm. While on the
127 south slope, the MAT varies from -5.65°C to 9.23°C with the average
128 temperature of 1.03°C , and the MAP varies from 124 mm to 308 mm with the
129 average precipitation of 246 mm. There were four meteorological observatories
130 along our elevation transects, two on either slope of Mount Tianshan (Table 1).

131 Fig. 1

132 Table 1

133

134 Intact and continuous vertical vegetation and soil spectrums can be observed along
135 the two slopes. On the north slope from bottom to top, vegetation spectrum consists
136 of upland desert (800–1100 m), upland steppe (1100–2500 m), frigid coniferous
137 forest (1800–2700 m), subalpine meadow (2500–3300 m), alpine meadow (3000–
138 3700 m), alpine sparse vegetation and a desert zone (3700–3900 m), and an alpine
139 ice-and-snow zone (> 3900 m). The main species on the north slope included
140 *Kobresia myosuroides*, *Carex enervis*, *Poa annua* and *Thalictrum aquilegifolium*. A
141 corresponding soil spectrum on the north slope includes brown calcic soil (800–1100
142 m), chestnut soil (1100–2500 m), mountain grey cinnamon forest soil (1800–2700
143 m), subalpine meadow soil (2500–3300 m), alpine meadow soil (3000–3700 m) and
144 chilly desert soil (> 3700 m). While on the south slope, it includes upland desert
145 (1300–1800 m), arid upland steppe (1800–2600 m), subalpine steppe (2600–2800 m),
146 alpine meadow and cushion plants (2800–3800 m), an alpine desert zone (3800–
147 4000 m), and an alpine ice-and-snow zone (> 4000 m). The main species occurred
148 on the south slope were *Ephedra sinica*, *Stipa grandis*, *Stipa capillata*, *Achnatherum*
149 *splendens*, *Nitraria tangutorum*, *Caragana sinica*, and *Suaeda glauca*. The
150 corresponding soil spectrum of the south slope consists of sierozem (1300–1800 m),
151 chestnut soil (1800–2800 m), alpine meadow soil (2800–3800 m), and chilly desert
152 soil (> 3800 m).

153

154 2.2 Plants and soil sampling

155 An altitudinal transect of 1,564 to 3,800 m above sea level (a.s.l.) was set on the
156 north slope, and 1,300 to 3,780 m a.s.l. on the south slope. Few human habitats
157 distribute along the two transects. Plant and soil samples were collected in July of
158 2014. To minimize the influences of human activities, light regime, or location
159 within the canopy, the sampling was restricted to open sites that are far from the
160 major roads and human habitats.

161 Plants and soil were collected along the two transects at altitudinal intervals of
162 about 100 m. Almost all plant species that we found at each sampling site were
163 collected, and at each site, 5–7 individual plants of each species were collected and
164 the same number of leaves was sampled from each individual plant. For shrubs and
165 herbs, the uppermost leaves of each individual plant were collected; for tree species,
166 8 leaves were collected from each individual, 2 leaves were collected at each of the 4
167 cardinal directions from the positions of full irradiance, about 8–10 m above the
168 ground. The leaves from the same species of each site were combined into one
169 sample. Excluding N-fixing plants and mosses, a total of 90 plant samples were
170 collected from north slope, including 72 herbs and 18 woody plants; 105 on the
171 south slope, covering 85 herbs and 20 woody plants.

172 Surface soils (0–5cm) were collected after removing the litter layer at each
173 sampling site. At each location, one composite soil sample was prepared by
174 combining six subsamples randomly taken within a radius of 20 m. Sample was used
175 to determine soil index including $\delta^{15}\text{N}$, N content, silt/clay ratio, pH and particle size.
176 In addition, at each sampling site, we also collected another three soil samples using

177 a ring, which were used to measure soil bulk density and moisture.

178

179 **2.3 Laboratory measurements**

180 Plant and soil samples were air-dried in the field and then in the laboratory. The soil

181 samples were sieved through a 2 mm sieve to remove stones and plant residues.

182 Plant leaves and about 5 g sieved soil samples were then ground into a fine powder

183 using a steel ball mixer mill MM200 (Retsch GmbH, Haan, Germany). $\delta^{15}\text{N}$, N and

184 C contents in leaves, and $\delta^{15}\text{N}$ and N contents in soil were measured on a Delta^{Plus}

185 XP mass spectrometer (Thermo Scientific, Bremen, Germany) coupled with an

186 automated elemental analyzer (Flash EA1112, CE Instruments, Wigan, UK) in a

187 continuous flow mode at the Stable Isotope Laboratory of the College of Resources

188 and Environmental Sciences, China Agricultural University. For this measurement,

189 we obtained standard deviations of less than 0.1% for C and N contents and less than

190 0.15‰ for $\delta^{15}\text{N}$ among replicates of the same sample.

191 The measurements of soil pH and soil particle size (clay, silt and sand content)

192 were determined using the sieved soil samples. Soil pH was measured using the pH

193 electrode in soil water suspension, with soil to water ratio of 1:2.5 (10 g soil and 25

194 mL deionized water removing carbon dioxide). Soil particle size (clay, silt and sand

195 content) was analyzed using a particle size analyzer (Malvern Masterizer 2000, UK)

196 after removing the calcium carbonates and organic matter. Soil moisture and bulk

197 density were determined after oven drying at 105 ± 2 °C to a constant weight. Soil

198 moisture of each sample was the difference between its wet and dry weight divided

199 by its dry weight. Soil bulk density was the dry weight divided by the certain volume
200 of the ring.

201

202 **2.4 Statistical analysis**

203 The MAT and MAP data of each sampling elevation used in the statistical analyses
204 were generated by interpolation based on the climatic data derived from the four
205 meteorological observatories distributed along the altitudinal transect. Statistical
206 analyses were conducted by SPSS software (SPSS for Windows, Version 20.0,
207 Chicago, IL, USA). One-way analysis variance (ANOVA) was used to compare leaf
208 $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$ and $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ between the north and south slopes. Leaf C/N was
209 ln- transformed to improve data normality. The relationships between $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$
210 and leaf $\delta^{15}\text{N}$ were performed by the linear regression on the two slopes. Leaf and
211 soil $\delta^{15}\text{N}$ were firstly analyzed by multiple linear regressions against all potential
212 influential factors using ordinary least square (OLS) estimation. The potential
213 influential factors of leaf $\delta^{15}\text{N}$ included MAP, MAT, leaf N content, leaf C/N, soil
214 $\delta^{15}\text{N}$, soil N content, silt/clay ratio, soil moisture, soil bulk density and soil pH. The
215 potential influential factors of soil $\delta^{15}\text{N}$ consisted of MAP, MAT, soil N content,
216 silt/clay ratio, soil moisture and soil bulk density and soil pH. Finally, both principal
217 component analysis (PCA) and correlation analysis were conducted to explore the
218 complicated relationship among these factors and leaf or soil $\delta^{15}\text{N}$.

219

220 **3 Results**

221 **3.1 Comparisons of $\delta^{15}\text{N}$ in leaf and soil between the north and the south slopes**

222 On Mount Tianshan, for all species pooled together, the arithmetic mean (mean \pm SE)
223 of leaf $\delta^{15}\text{N}$ were $0.5 \pm 0.2\text{‰}$ and $2.0 \pm 0.2\text{‰}$ for the plants grown on the north and
224 the south slopes, respectively. One-way ANOVA suggested a significant difference
225 for leaf $\delta^{15}\text{N}$ between the north and south slopes ($P < 0.001$) (Fig. 2a). The mean soil
226 $\delta^{15}\text{N}$ of the north and south slope were $4.1 \pm 0.4\text{‰}$ and $5.0 \pm 0.8\text{‰}$, respectively.
227 One-way ANOVA showed that sampling slope exerted no significant effect on soil
228 $\delta^{15}\text{N}$ ($P = 0.290$) (Fig. 2b). The mean $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ was $-3.6 \pm 0.3\text{‰}$ for the north
229 slope and $-2.4 \pm 0.3\text{‰}$ for the south slope, and one-way ANOVA suggested a
230 significant difference in $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ between the two slopes ($P = 0.003$) (Fig. 2c). In
231 addition, this study showed that $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ was positively related to $\delta^{15}\text{N}_{\text{leaf}}$ on the
232 two slopes ($P < 0.001$, Fig. 3).

233 Fig. 2

234 Fig. 3

235

236 **3.2 The relationships between leaf $\delta^{15}\text{N}$ and potential influential factors**

237 A multiple regression of leaf $\delta^{15}\text{N}$ against potential influential factors including soil
238 $\delta^{15}\text{N}$, MAT, MAP, leaf N content, leaf C/N, soil N content, soil moisture, soil pH,
239 soil bulk density and silt/clay was conducted. The statistical analyses showed that
240 45.8% and 23.4% of the variability in leaf $\delta^{15}\text{N}$ on the north slope and south slope
241 could be explained as a linear combination of all 10 independent variables,
242 respectively ($P < 0.001$ for the north slope and $P = 0.005$ for the south slope) (Table

243 2). Among these influential factors, MAT, leaf N content correlated positively and
244 leaf C/N, MAP, soil moisture and silt/clay ratio correlated negatively with leaf $\delta^{15}\text{N}$
245 on the north slope (Table 3). The results of PCA also showed that leaf N content had
246 strong positive while leaf C/N had negative effects on leaf $\delta^{15}\text{N}$, MAT and MAP also
247 exerted influences on leaf $\delta^{15}\text{N}$, however, soil factors almost did not affect leaf $\delta^{15}\text{N}$
248 except silt/clay ratio and soil moisture (Fig. 4a). Whereas on the south slope, only
249 leaf C/N was found to have a negative effect on leaf $\delta^{15}\text{N}$, MAP correlated
250 marginally and negatively with leaf $\delta^{15}\text{N}$ (Table 4). Besides, on the south slope, PC1
251 and PC2 could almost represent soil conditions and plant traits, respectively, leaf
252 $\delta^{15}\text{N}$ was affected strongly by leaf C/N (Fig. 4b).

253 Table 2

254 Table 3

255 Table 4

256 Fig. 4

257

258 **3.3 The relationships between soil $\delta^{15}\text{N}$ and potential influential factors**

259 Multiple regressions analysis with soil $\delta^{15}\text{N}$ as a dependent variable and MAT, MAP,
260 soil N content, silt/clay ratio, soil moisture, soil bulk density and soil pH as
261 independent variables were conducted separately for the north slope and south slope.
262 The statistical analyses showed that the regressions were very significant on both
263 slopes ($P < 0.001$ for the both slopes). The seven factors in total accounted for 55.2%
264 and 72.7% of soil $\delta^{15}\text{N}$ variance on the north and south slope, respectively (Table 2).

265 Considering the potential link between soil N and plant N, new multiple regressions
266 including leaf $\delta^{15}\text{N}$, leaf N and leaf C/N were performed on the two slopes.
267 Compared to the old multiple regressions, the new regressions did not exhibit
268 changes in R^2 and P values on both slopes (in the new regressions $P < 0.001$ and R^2
269 $= 0.563$ for the north slope, $P < 0.001$ and $R^2 = 0.738$ for the south slope).
270 Furthermore, compared to the adjusted R^2 values derived from the old regressions
271 (adjusted $R^2 = 0.513$ for the north slope, adjusted $R^2 = 0.708$ for the south slope), the
272 values of the new regressions were smaller or almost unchanged (adjusted $R^2 =$
273 0.506 for the north slope, adjusted $R^2 = 0.709$ for the south slope) (Table 2). Thus,
274 the new multiple regressions indicated no effect of leaf nutrient traits on soil $\delta^{15}\text{N}$.
275 Among these factors, MAT, MAP, soil moisture and silt/clay were found to be
276 significantly related to the soil $\delta^{15}\text{N}$ of the north slope (Table 3). The PCA showed
277 that both MAT and MAP had large loadings on soil $\delta^{15}\text{N}$, meanwhile, soil $\delta^{15}\text{N}$
278 increased with decreasing silt/clay ratio and increasing soil moisture (Fig. 4a).
279 However, on the south slope, only MAP and soil moisture were found to play a
280 significant and negative role in soil $\delta^{15}\text{N}$ (Table 4, Fig. 4b).

281

282 **4 Discussion**

283 **4.1 Differences in leaf $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$ and $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ between the south and the** 284 **north slopes**

285 On Mount Tianshan, leaf $\delta^{15}\text{N}$ and $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ both showed higher values on the
286 south slope than the north slope; soil $\delta^{15}\text{N}$ of the south slope was also more positive

287 than that of the north slope although the difference was not significant. The results
288 confirmed our hypothesis that, for a given mountain, the leaf $\delta^{15}\text{N}$, soil $\delta^{15}\text{N}$ and
289 $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ of the south slope could differ from those of the north slope. Greater leaf
290 $\delta^{15}\text{N}$ on the south slope than north slope suggested that the south slope had higher
291 soil N availability and higher soil N transformation rates (N mineralization or
292 nitrification rates) (Garten and Van Miegroet, 1994; McLauchlan et al., 2007).
293 Increasing soil N transformation rates led to an increase in soil available N.
294 Meanwhile, increasing soil N transformation rates could result in more ^{15}N
295 enrichment in soil available N sources, and consequently plant $\delta^{15}\text{N}$ increased,
296 because N transformation processes discriminate against ^{14}N .

297 $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ was greater on the south slope than the north slope. This result also
298 suggested that the south slope has higher N availability and N mineralization or
299 nitrification rates relative to the north slope, because previous studies reported that
300 $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ increased with increasing soil N transformation rates (N mineralization
301 or nitrification rates) and N availability (Garten and Van Miegroet, 1994; Kahmen et
302 al., 2008; Cheng et al., 2010).

303 Amundson et al. (2003) suggested that $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ could be interpreted as the
304 isotopic composition of plant-available N provided that isotopic discrimination does
305 not occur during plant uptake and assimilation. In the present study, we found a
306 highly correlation between leaf $\delta^{15}\text{N}$ and $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ on each slope, which was
307 consistent with the result observed by Craine et al. (2009). Additionally, leaf $\delta^{15}\text{N}$
308 has been widely considered as a good approximation to $\delta^{15}\text{N}$ of soil available

309 nitrogen sources in natural ecosystems (Virginia and Delwiche, 1982; Cheng et al.,
310 2010; Craine et al., 2015a), because N isotopic discrimination would be negligible
311 during nitrogen uptake and assimilation due to limited soil available N in most
312 natural ecosystems (Högeberg et al., 1999). Thus, the significant correlated
313 relationship between leaf $\delta^{15}\text{N}$ and $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ could provide a powerful support for
314 the suggestion in Amundson et al. (2003).

315

316 **4.2 Influences of various factors on leaf $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$: south slope versus** 317 **north slope**

318 The regression and correlation analyses showed that each factor did not exert
319 completely identical effect on leaf $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ across the two slopes, this
320 provided powerful support for our argument that the influences of environmental
321 factors on leaf $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ are localized.

322 Leaf C/N may play a role in regulating biogeochemical cycles of carbon and
323 nitrogen in natural ecosystems (Luo et al., 2004), or, conversely, soil
324 biogeochemistry and plant physiology also cause the shifts in leaf C/N
325 stoichiometric characters (Reich and Oleksyn, 2004; Yang et al., 2011). In this study,
326 leaf C/N was negatively correlated with leaf $\delta^{15}\text{N}$ on both slopes of Mount Tianshan.
327 The result was similar to the finding by Pardo et al. (2006), in which leaf $\delta^{15}\text{N}$ and
328 root $\delta^{15}\text{N}$ both decreased with forest floor C/N. A negative correlation between leaf
329 C/N and $\delta^{15}\text{N}$ was also reported for the fine roots in Glacier Bay (Hobbie et al.,
330 2000). Two possible reasons were responsible for the pattern observed in the present

331 study. First, the increase in leaf C/N might be caused by enhanced photosynthesis,
332 which would aggravate the limit in nitrogen nutrients and result in a decrease in
333 nitrogen availability. As we all know, when soil N availability is high and N nutrient
334 is rich, soil N transformations, such as NH₃ volatilization and NO_x emission are
335 enhanced, consequently, more ¹⁴N losses from soil. This causes ¹⁵N enrichment in
336 soil, subsequently, plant δ¹⁵N is more positive. Conversely, plants have more
337 negative δ¹⁵N values when soil N is limited because of weak soil N transformations
338 and less ¹⁴N loss. Thus, an increase in leaf C/N caused a decrease in nitrogen
339 availability and ¹⁵N-depletion in plants. Second, leaf C/N usually was considered to
340 be negatively correlated with leaf N contents because leaf C contents always keep
341 relative stable (Tan and Wang, 2016). The relative stability of leaf C was also
342 observed in this study. The negative relationship between leaf C/N and leaf δ¹⁵N
343 might be caused by the positive relationship between leaf N content and leaf δ¹⁵N,
344 which has been reported by many studies (Chen et al., 2015; Zhang et al., 2015;
345 Craine et al., 2012; Pardo et al., 2006; Craine et al., 2009; Martinelli et al., 1999).
346 This study also found a positive relationship between leaf N content and leaf δ¹⁵N on
347 the north slope of Mount Tianshan.

348 MAP was observed to be significantly and negatively correlated with leaf δ¹⁵N on
349 the north slope; however, on the south slope the relationship was just marginally
350 significant. A negative relationship between leaf δ¹⁵N and MAP was reported in
351 many previous studies (Austin and Sala, 1999; Handley et al., 1999; Robinson, 2001;
352 Amundson et al., 2003; Craine et al., 2009). The decrease in leaf δ¹⁵N with

353 increasing precipitation could be associated with decreased gaseous N loss in wetter
354 regions (Houlton et al., 2006).

355 MAT played a positive effect in the leaf $\delta^{15}\text{N}$ of the north slope, which was
356 consistent with many previous studies (Amundson et al., 2003; Craine et al., 2009);
357 whereas on the south slope the effect of MAT was not observed. The probable
358 explanation for the observations was that climate on the north slope is very cold (the
359 average MAT = $-1.85\text{ }^{\circ}\text{C}$), temperature is the key growth-limiting factor for plants.
360 Previous studies consistently suggested that the key factor limiting plant growth
361 generally also plays a dominant role in plant isotope discrimination (McCarroll and
362 Loader, 2004; Winter et al., 1982; Xu et al., 2015), thus, temperature exerted an
363 effect on leaf $\delta^{15}\text{N}$. However, on the south slope, climate is relatively warm except
364 those sites with higher altitudes, and usually, temperature does not limit plant growth,
365 thus, leaf $\delta^{15}\text{N}$ was not related to temperature. With respect to the positive effect of
366 temperature on the north slope, the mechanism might be that higher temperature
367 favors more complete plant nitrogen assimilation and transformation, this might
368 decrease isotopic fractionation during N assimilation and transformation, then cause
369 ^{15}N enrichment in plants.

370 On Mount Tianshan, soil $\delta^{15}\text{N}$ increased with increasing MAP on the north slope,
371 while decreased with increasing MAP on the south slope. Soil $\delta^{15}\text{N}$ could be
372 determined by the balance of the N input or output processes and corresponding
373 isotopic fractionation factors (Brenner et al., 2001; Bai and Houlton, 2009; Wang et
374 al., 2014). Considering that the leaching loss could be neglected on both slopes

375 because of the dry environment, soil $\delta^{15}\text{N}$ can be estimated by the following
376 equation:

$$377 \quad \text{Soil } \delta^{15}\text{N} = \delta^{15}\text{N}_{\text{input}} + \varepsilon_{\text{G}} \times f_{\text{G}} + \varepsilon_{\text{P}} \times f_{\text{P}} \quad (1)$$

378 where $\delta^{15}\text{N}_{\text{input}}$ is the input $\delta^{15}\text{N}$; f_{G} and f_{P} are the fraction of gas losses and net plant
379 N accumulation out of total N losses (%), respectively; ε_{G} and ε_{P} are the
380 fractionation factors of corresponding N losses processes, respectively. And

$$381 \quad f_{\text{G}} + f_{\text{P}} = 1 \quad (2)$$

382 ε_{G} varies from 16‰ to 30‰ (Handley et al., 1999; Robinson, 2001); ε_{P} is between 5‰
383 and 10‰ (Handley et al., 1999; Evans, 2001), thus, in general, $\varepsilon_{\text{G}} > \varepsilon_{\text{P}}$, and soil $\delta^{15}\text{N}$
384 is correlated positively with f_{G} and negatively with f_{P} based on eqn. (1) and (2). On
385 the north slope, rainfall event may accelerate the gas losses (nitrification and
386 denitrification processes) more than plant N uptake, while it may be opposite on the
387 south slope. On the north slope, with increase in MAP, f_{G} increases and causes ^{15}N
388 enrichment in soil; on the south slope, f_{P} increases with MAP, and results in ^{15}N
389 depletion in soil.

390 The effects of silt/clay ratio on soil $\delta^{15}\text{N}$ might be driven by the indirect effects of
391 silt/clay ratio on soil moisture and soil oxygen concentrations. The north slope is
392 wetter than the south slope, and the north slope will prefer denitrification, while
393 nitrification will be favored on the south slope. On the north slope, with increase in
394 silt/clay ratio, soil oxygen concentration increases and this inhibits soil
395 denitrification, consequently, ^{15}N depletion in soil would be resulted in, thus silt/clay
396 ratio showed a negative relationship with soil $\delta^{15}\text{N}$.

397

398 **5 Conclusion**

399 We sampled plants and soils on the south slope and north slope of Mount Tianshan
400 and measured their $\delta^{15}\text{N}$. South slope differs significantly in climate and
401 environment from north slope. In the present study, leaf $\delta^{15}\text{N}$ and $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ (leaf
402 $\delta^{15}\text{N}$ – soil $\delta^{15}\text{N}$) of the south slope were more positive than that of the north slope,
403 soil $\delta^{15}\text{N}$ of the south slope was also higher than that of the north slope although the
404 difference between the two slopes was not significant. The results suggested that the
405 south slope has higher soil N transformation rates and soil N availability relative to
406 the north slope. In addition, among the potential influential factors, MAP, leaf C/N,
407 soil moisture and silt/clay ratio had negative effects while MAT and leaf N content
408 had positive effects on leaf $\delta^{15}\text{N}$ of the north slope; however, on the south slope, only
409 leaf C/N played a negative role in leaf $\delta^{15}\text{N}$. For soil $\delta^{15}\text{N}$, the significant influential
410 factors were MAT, MAP, soil moisture and silt/clay ratio on the north slope, whereas
411 on the south slope, MAP and soil moisture exerted significant effects. Interestingly,
412 MAP was found to exert contrary effects on soil $\delta^{15}\text{N}$ between the two slopes. This
413 indicated that environmental influences on leaf $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ are localized.

414

415 *Data availability.* There is no underlying material and related items in this paper. All
416 data are provided in the Supplement.

417

418 *Competing financial interests.* The authors declare no competing financial interests.

419

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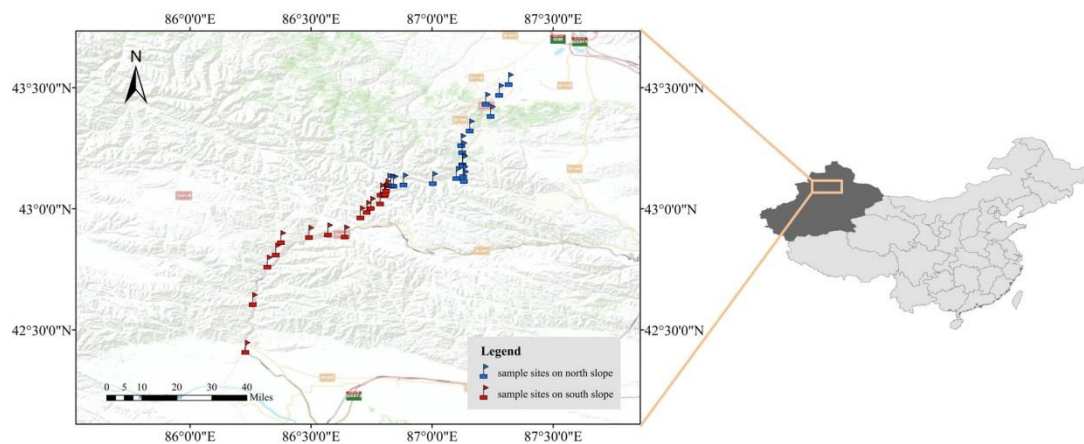
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Figures

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572 **Fig 1. Sketch of study area. Locations of the sampling sites are indicated with points. A total**
573 **of 17 sites (blue dots) were selected on the north slope, and 16 sites (red dots) on the south**
574 **slope.**

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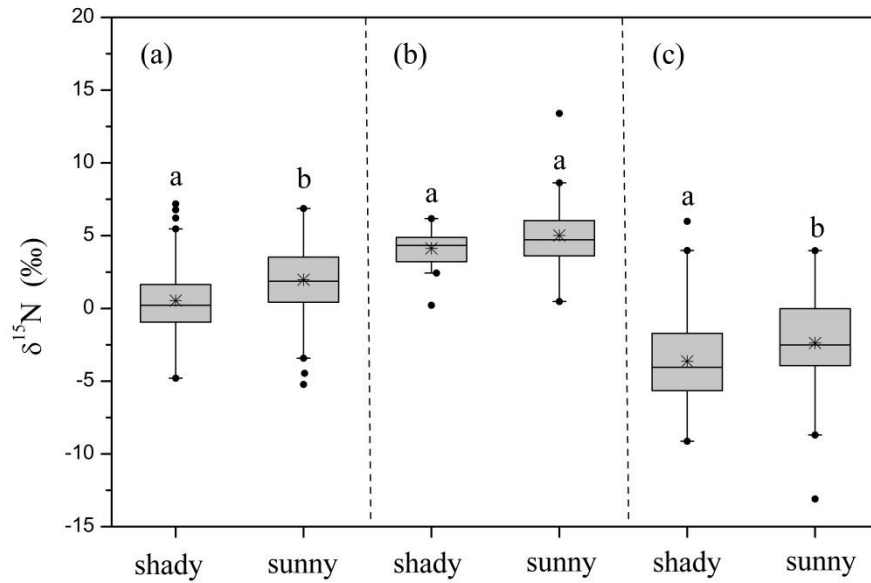
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585 **Fig 2. Differences in (a) leaf $\delta^{15}\text{N}$, (b) soil $\delta^{15}\text{N}$ and (c) $\Delta\delta^{15}\text{N}_{\text{leaf-soil}}$ between the north and**

586 **south slopes of Mount Tianshan.** Each box represents range of middle 50% of group values, the

587 center lines and points within the boxes are median and mean values. Whiskers are outside 25%

588 each, and dots are outliers.

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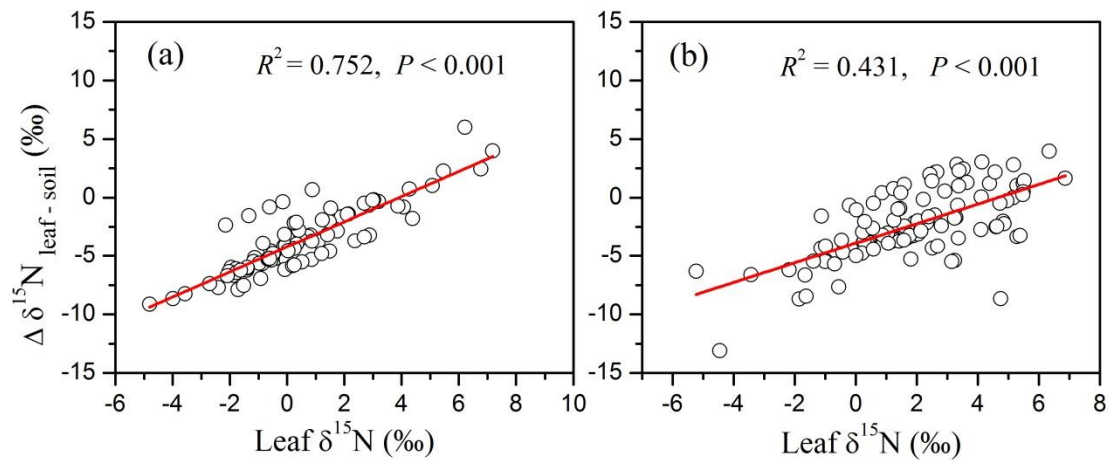
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601 **Fig 3. Relationships between $\Delta \delta^{15}\text{N}_{\text{leaf-soil}}$ and leaf $\delta^{15}\text{N}$ on the north slope (a) and the south**

602 **slope (b) of Mount Tianshan.**

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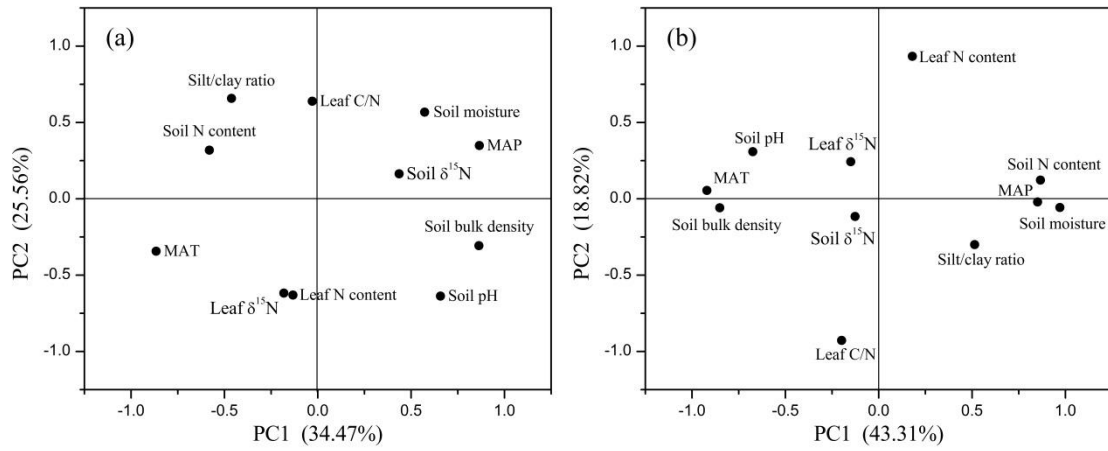
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619 **Fig 4. Variables loading on the first two principle components of the north (a) and south**

620 **slope (b) of Mount Tianshan.**

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Tables

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637 **Table 1. Climate data from the meteorological observatories in the research area**

Meteorological observatories	Locations	MAT/°C	MAP/mm	Alt./m
WLMQ	north slope	6.9	269.4	918.7
MOS	north slope	-5.2	453.4	3539.0
BLT	south slope	6.6	208.4	1738.3
YQ	south slope	8.4	73.3	1055.8

638 *Abbreviation: WLMQ, Wulumuqi Meteorological Observatory; MOS, Mountain Observation*

639 *Station of the Tianshan Glaciological Station, Chinese Academy of Sciences; BLT, Baluntai*

640 *Meteorological Observatory; YQ, Yanqi Meteorological Observatory; MAT, annual mean*

641 *temperature; MAP, annual mean precipitation.*

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652 **Table 2. Multiple linear regressions of leaf $\delta^{15}\text{N}$ and soil $\delta^{15}\text{N}$ based on ordinary least**
653 **square (OLS) estimation.**

Model	Dependent variable	North slope			South slope		
		R^2	Adjusted R^2	P	R^2	Adjusted R^2	P
1	Leaf $\delta^{15}\text{N}$	0.458	0.388	< 0.001	0.234	0.150	0.005
2	Soil $\delta^{15}\text{N}$	0.552	0.513	< 0.001	0.727	0.708	< 0.001
3	Soil $\delta^{15}\text{N}$	0.563	0.506	< 0.001	0.738	0.709	< 0.001

654 *Note: In the model-1, independent variables were MAT, MAP, leaf N content, leaf C/N, soil $\delta^{15}\text{N}$,*
655 *soil N content, silt/clay, soil moisture, soil bulk density and soil pH. In the model-2, independent*
656 *variables were MAT, MAP, soil N content, silt/clay, soil moisture, soil bulk density and soil pH.*
657 *In the model-3, besides all variables in Model-2, leaf $\delta^{15}\text{N}$, leaf N content and leaf C/N were also*
658 *included in independent variables.*

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670 **Table 3. Correlation analyses between leaf or soil $\delta^{15}\text{N}$ and influential factors on the north**

671 **slope of Mount Tianshan.**

	Leaf $\delta^{15}\text{N}$		Soil $\delta^{15}\text{N}$	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Leaf $\delta^{15}\text{N}$	1	---	-0.120	0.264
Soil $\delta^{15}\text{N}$	-0.120	0.264	1	---
MAT	0.266	0.012	-0.385	< 0.001
MAP	-0.272	0.010	0.387	< 0.001
Leaf N content	0.340	0.001	-0.090	0.397
Leaf C/N	-0.452	< 0.001	-0.036	0.739
Soil N content	-0.048	0.659	0.088	0.408
Soil moisture	-0.271	0.011	0.388	0.000
Soil pH	0.162	0.132	0.070	0.513
Soil bulk density	-0.056	0.604	0.145	0.174
Silt/clay ratio	-0.236	0.027	-0.370	< 0.001

672 *Note: the r values were in bold when $P < 0.05$.*

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684 **Table 4. Correlation analyses between leaf or soil $\delta^{15}\text{N}$ and influential factors on the south**
685 **slope of Mount Tianshan.**

	Leaf $\delta^{15}\text{N}$		Soil $\delta^{15}\text{N}$	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Leaf $\delta^{15}\text{N}$	1	---	0.175	0.074
Soil $\delta^{15}\text{N}$	0.175	0.074	1	---
MAT	0.157	0.109	0.115	0.244
MAP	-0.168	0.087	-0.203	0.038
Leaf N content	0.119	0.229	-0.073	0.459
Leaf C/N	-0.228	0.021	0.062	0.533
Soil N content	-0.173	0.078	0.014	0.888
Soil moisture	-0.141	0.150	-0.229	0.019
Soil pH	0.04	0.686	-0.138	0.161
Soil bulk density	0.151	0.125	0.041	0.679
Silt/clay ratio	-0.07	0.477	-0.004	0.964

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Note: the r values were in bold when $P < 0.05$.

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