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

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Abstract

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

47 study supported our argument that the relationships between leaf and soil δ^{15}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 (δ^{15}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 δ^{15}N

61 and soil δ^{15}N ($\Delta\delta^{15}$N$_{leaf-soil}$ = leaf δ^{15}N – soil δ^{15}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 $\delta^{15}N$, soil $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf-soil}$

74   could reflect and indicate ecosystem N cycling, differences in leaf $\delta^{15}N$, soil $\delta^{15}N$

75   and $\Delta\delta^{15}N_{leaf-soil}$ were expected to appear between south and north slopes.

76   Comparisons on leaf $\delta^{15}N$, soil $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf-soil}$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$, soil $\delta^{15}N$ has been little addressed. Some

87   studies demonstrated that soil $\delta^{15}N$ 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 $\delta^{15}N$ increased with decreasing MAT

91   in Inner Mongolian. Sheng et al. (2014) showed that the soil $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ or soil $\delta^{15}N$ would depend on local environment.

98   Comparisons on the effects of climatic and environmental factors on leaf $\delta^{15}N$ and

99   soil $\delta^{15}N$ 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 $\delta^{15}N$, soil $\delta^{15}N$

106 and $\Delta\delta^{15}N_{leaf-soil}$. The second objective was to test our argument that environmental

107 effects on leaf $\delta^{15}N$ and soil $\delta^{15}N$ 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 ($42.43\degree - 43.53\degree$N, $86.23\degree - 87.32\degree$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 ℃ to 3.90 ℃ with the

125   average temperature of -1.85 ℃, 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 ℃ to 9.23 ℃ with the average

128   temperature of 1.03 ℃, 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

Intact and continuous vertical vegetation and soil spectrums can be observed along

the two slopes. On the north slope from bottom to top, vegetation spectrum consists

of upland desert (800–1100 m), upland steppe (1100–2500 m), frigid coniferous

forest (1800–2700 m), subalpine meadow (2500–3300 m), alpine meadow (3000–

3700 m), alpine sparse vegetation and a desert zone (3700–3900 m), and an alpine

ice-and-snow zone (> 3900 m). The main species on the north slope included

Kobresia myosuroides, *Carex enervis, Poa annua* and *Thalictrum aquilegifolium*. A

corresponding soil spectrum on the north slope includes brown calcic soil (800–1100

m), chestnut soil (1100–2500 m), mountain grey cinnamon forest soil (1800–2700

m), subalpine meadow soil (2500–3300 m), alpine meadow soil (3000–3700 m) and

chilly desert soil (> 3700 m). While on the south slope, it includes upland desert

(1300–1800 m), arid upland steppe (1800–2600 m), subalpine steppe (2600–2800 m),

alpine meadow and cushion plants (2800–3800 m), an alpine desert zone (3800–

4000 m), and an alpine ice-and-snow zone (> 4000 m). The main species occurred

on the south slope were *Ephedra sinica*, *Stipa grandis*, *Stipa capillata*, *Achnatherum*

splendens, *Nitraria tangutorum*, *Caragana sinica*, and *Suaeda glauca*. The

corresponding soil spectrum of the south slope consists of sierozem (1300–1800 m),

chestnut soil (1800–2800 m), alpine meadow soil (2800–3800 m), and chilly desert

soil (> 3800 m).

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 $\delta^{15}N$, 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). $\delta^{15}N$, N and

184 C contents in leaves, and $\delta^{15}N$ 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 $\delta^{15}N$ 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 ℃ 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   δ^{15}N, soil δ^{15}N and $\Delta\delta^{15}$N$_{\text{leaf-soil}}$ between the north and south slopes. Leaf C/N was

209   ln- transformed to improve data normality. The relationships between $\Delta\delta^{15}$N$_{\text{leaf-soil}}$

210   and leaf δ^{15}N were performed by the linear regression on the two slopes. Leaf and

211   soil δ^{15}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 δ^{15}N included MAP, MAT, leaf N content, leaf C/N, soil

214   δ^{15}N, soil N content, silt/clay ratio, soil moisture, soil bulk density and soil pH. The

215   potential influential factors of soil δ^{15}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 δ^{15}N.

219

220   **3 Results**

3.1 Comparisons of δ¹⁵N in leaf and soil between the north and the south slopes

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

Fig. 2

Fig. 3

3.2 The relationships between leaf δ¹⁵N and potential influential factors

A multiple regression of leaf $\delta^{15}N$ against potential influential factors including soil $\delta^{15}N$, MAT, MAP, leaf N content, leaf C/N, soil N content, soil moisture, soil pH, soil bulk density and silt/clay was conducted. The statistical analyses showed that 45.8% and 23.4% of the variability in leaf $\delta^{15}N$ on the north slope and south slope could be explained as a linear combination of all 10 independent variables, 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 δ^{15}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 δ^{15}N, MAT and MAP also

247 exerted influences on leaf δ^{15}N, however, soil factors almost did not affect leaf δ^{15}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 δ^{15}N, MAP correlated

250 marginally and negatively with leaf δ^{15}N (Table 4). Besides, on the south slope, PC1

251 and PC2 could almost represent soil conditions and plant traits, respectively, leaf

252 δ^{15}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 δ^{15}N and potential influential factors**

259 Multiple regressions analysis with soil δ^{15}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 δ^{15}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 δ^{15}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 δ^{15}N.

275   Among these factors, MAT, MAP, soil moisture and silt/clay were found to be

276   significantly related to the soil δ^{15}N of the north slope (Table 3). The PCA showed

277   that both MAT and MAP had large loadings on soil δ^{15}N, meanwhile, soil δ^{15}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 δ^{15}N (Table 4, Fig. 4b).

281

282   **4 Discussion**

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

284   **north slopes**

285   On Mount Tianshan, leaf δ^{15}N and $\Delta\delta^{15}$N$_{\text{leaf-soil}}$ both showed higher values on the

286   south slope than the north slope; soil δ^{15}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 $\delta^{15}N$, soil $\delta^{15}N$ and

289 $\Delta\delta^{15}N_{leaf\text{-}soil}$ of the south slope could differ from those of the north slope. Greater leaf

290 $\delta^{15}N$ 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 $\delta^{15}N$ increased,

296 because N transformation processes discriminate against ^{14}N.

297 $\Delta\delta^{15}N_{leaf\text{-}soil}$ 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 $\Delta\delta^{15}N_{leaf\text{-}soil}$ 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 $\Delta\delta^{15}N_{leaf\text{-}soil}$ 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 $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf\text{-}soil}$ on each slope, which was

307 consistent with the result observed by Craine et al. (2009). Additionally, leaf $\delta^{15}N$

308 has been widely considered as a good approximation to $\delta^{15}N$ 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 $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf-soil}$ could provide a powerful support for

314   the suggestion in Amundson et al. (2003).

315

316   **4.2 Influences of various factors on leaf $\delta^{15}N$ and soil $\delta^{15}N$: 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 $\delta^{15}N$ and soil $\delta^{15}N$ across the two slopes, this

320   provided powerful support for our argument that the influences of environmental

321   factors on leaf $\delta^{15}N$ and soil $\delta^{15}N$ 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 $\delta^{15}N$ on both slopes of Mount Tianshan.

327   The result was similar to the finding by Pardo et al. (2006), in which leaf $\delta^{15}N$ and

328   root $\delta^{15}N$ both decreased with forest floor C/N. A negative correlation between leaf

329   C/N and $\delta^{15}N$ 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 NH_3 volatilization and NO_x emission are

335 enhanced, consequently, more ^{14}N losses from soil. This causes ^{15}N enrichment in

336 soil, subsequently, plant $\delta^{15}N$ is more positive. Conversely, plants have more

337 negative $\delta^{15}N$ values when soil N is limited because of weak soil N transformations

338 and less ^{14}N loss. Thus, an increase in leaf C/N caused a decrease in nitrogen

339 availability and ^{15}N-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 $\delta^{15}N$

343 might be caused by the positive relationship between leaf N content and leaf $\delta^{15}N$,

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 $\delta^{15}N$ on

347 the north slope of Mount Tianshan.

348   MAP was observed to be significantly and negatively correlated with leaf $\delta^{15}N$ on

349 the north slope; however, on the south slope the relationship was just marginally

350 significant. A negative relationship between leaf $\delta^{15}N$ 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 $\delta^{15}N$ 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 $\delta^{15}N$ 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 ℃), 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 $\delta^{15}N$. 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 $\delta^{15}N$ 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 $\delta^{15}N$ increased with increasing MAP on the north slope,

371   while decreased with increasing MAP on the south slope. Soil $\delta^{15}N$ 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 $\delta^{15}N$ can be estimated by the following

376 equation:

377 $$\text{Soil } \delta^{15}N = \delta^{15}N_{input} + \varepsilon_G \times f_G + \varepsilon_P \times f_P \qquad (1)$$

378 where $\delta^{15}N_{input}$ is the input $\delta^{15}N$; 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 $$f_G + f_P = 1 \qquad (2)$$

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, $\varepsilon_G > \varepsilon_P$, and soil $\delta^{15}N$

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 $\delta^{15}N$ 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 $\delta^{15}N$.

397

5 Conclusion

We sampled plants and soils on the south slope and north slope of Mount Tianshan

and measured their $\delta^{15}N$. South slope differs significantly in climate and

environment from north slope. In the present study, leaf $\delta^{15}N$ and $\Delta\delta^{15}N_{leaf\text{-}soil}$ (leaf

$\delta^{15}N$ – soil $\delta^{15}N$) of the south slope were more positive than that of the north slope,

soil $\delta^{15}N$ of the south slope was also higher than that of the north slope although the

difference between the two slopes was not significant. The results suggested that the

south slope has higher soil N transformation rates and soil N availability relative to

the north slope. In addition, among the potential influential factors, MAP, leaf C/N,

soil moisture and silt/clay ratio had negative effects while MAT and leaf N content

had positive effects on leaf $\delta^{15}N$ of the north slope; however, on the south slope, only

leaf C/N played a negative role in leaf $\delta^{15}N$. For soil $\delta^{15}N$, the significant influential

factors were MAT, MAP, soil moisture and silt/clay ratio on the north slope, whereas

on the south slope, MAP and soil moisture exerted significant effects. Interestingly,

MAP was found to exert contrary effects on soil $\delta^{15}N$ between the two slopes. This

indicated that environmental influences on leaf $\delta^{15}N$ and soil $\delta^{15}N$ are

local-dependent.

Data availability. There is no underlying material and related items in this paper. All

data will be provided in the Supplement.

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

420

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Figures

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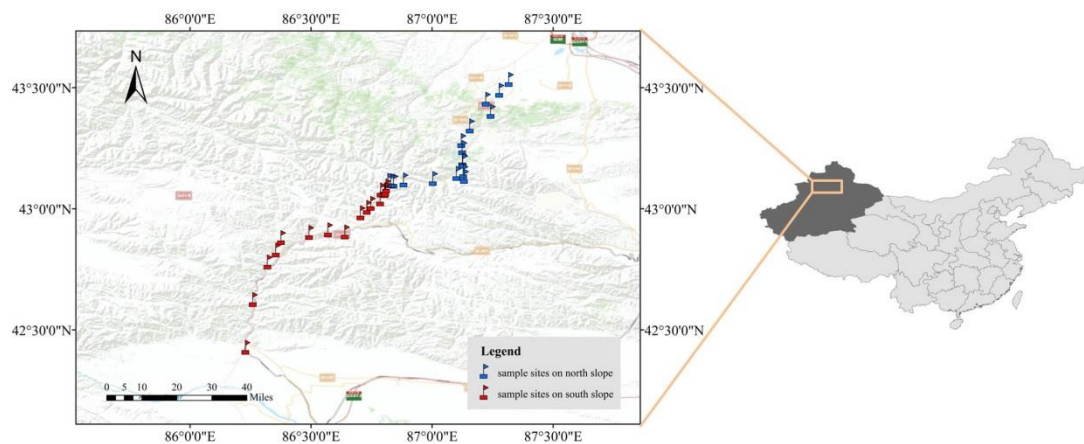
Fig 1. Sketch of study area. Locations of the sampling sites are indicated with points. A total of 17 sites (blue dots) were selected on the north slope, and 16 sites (red dots) on the south slope.

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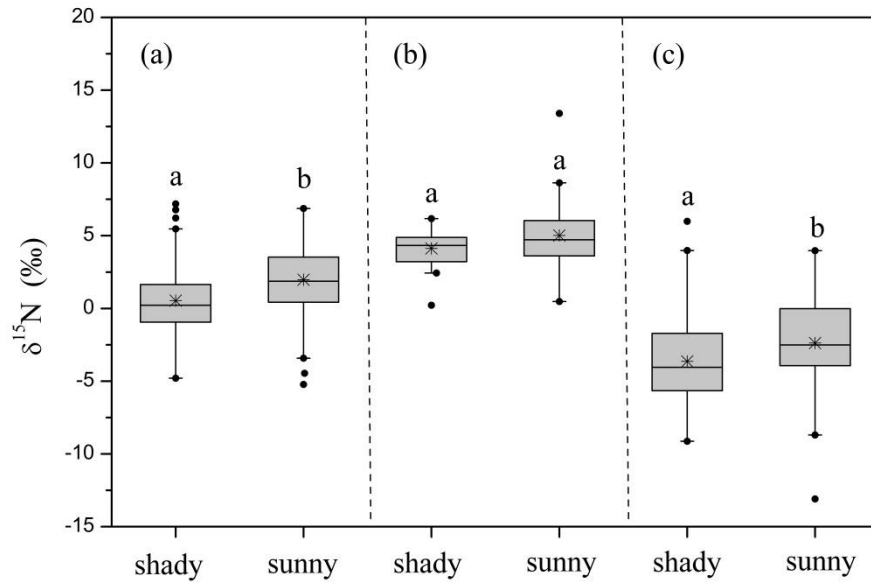
Fig 2. Differences in (a) leaf $\delta^{15}N$, (b) soil $\delta^{15}N$ and (c) $\Delta\delta^{15}N_{leaf-soil}$ between the north and south slopes of Mount Tianshan. Each box represents range of middle 50% of group values, the center lines and points within the boxes are median and mean values. Whiskers are outside 25% each, and dots are outliers.

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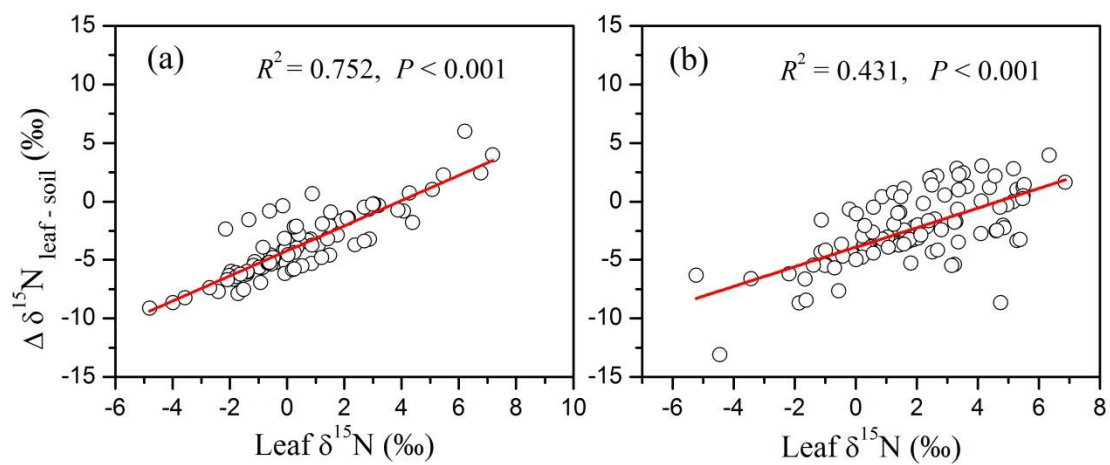
Fig 3. Relationships between $\Delta\delta^{15}N_{leaf\text{-}soil}$ and leaf $\delta^{15}N$ on the north slope (a) and the south

slope (b) of Mount Tianshan.

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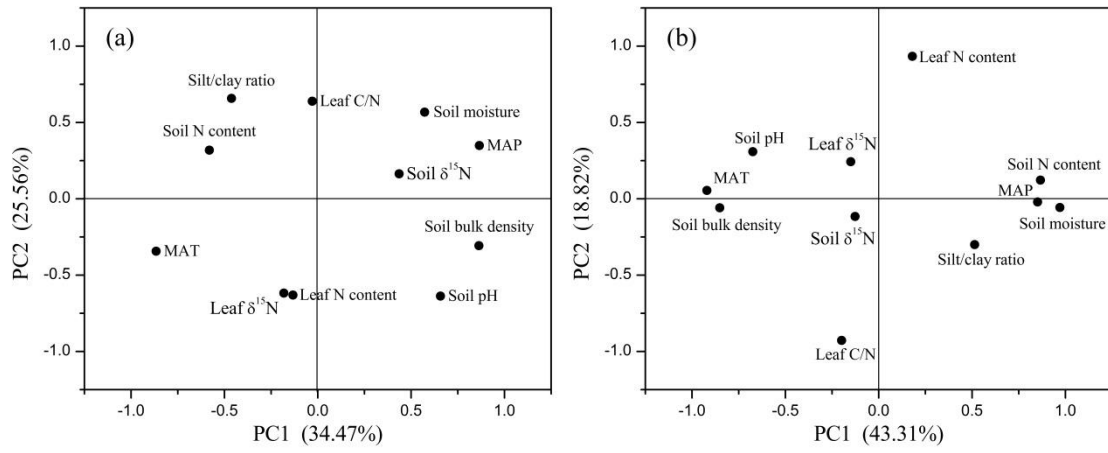
Fig 4. Variables loading on the first two principle components of the north (a) and south slope (b) of Mount Tianshan.

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Tables

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

Meteorological observatories	Locations	MAT/℃	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

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

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

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

642 *temperature; MAP, annual mean precipitation.*

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Table 2. Multiple linear regressions of leaf δ^{15}N and soil δ^{15}N based on ordinary least

square (OLS) estimation.

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

655 *Note: In the model-1, independent variables were MAT, MAP, leaf N content, leaf C/N, soil δ^{15}N,*

656 *soil N content, silt/clay, soil moisture, soil bulk density and soil pH. In the model-2, independent*

657 *variables were MAT, MAP, soil N content, silt/clay, soil moisture, soil bulk density and soil pH.*

658 *In the model-3, besides all variables in Model-2, leaf δ^{15}N, leaf N content and leaf C/N were also*

659 *included in independent variables.*

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Table 3. Correlation analyses between leaf or soil δ^{15}N and influential factors on the north slope of Mount Tianshan.

	Leaf δ^{15}N		Soil δ^{15}N	
	r	*P*	*r*	*P*
Leaf δ^{15}N	1	---	-0.120	0.264
Soil δ^{15}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

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

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Table 4. Correlation analyses between leaf or soil δ¹⁵N and influential factors on the south slope of Mount Tianshan.

	Leaf δ^{15}N		Soil δ^{15}N	
	r	*P*	*r*	*P*
Leaf δ^{15}N	1	---	0.175	0.074
Soil δ^{15}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

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

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