

The effects of additional nitrogen and phosphorus on ecosystem nitrogen retention: a case study in an Alpine Meadow on the Qinghai-Tibet Plateau, China.

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1 The effects of additional nitrogen and phosphorus on ecosystem nitrogen retention: a
2 case study in an Alpine Meadow on the Qinghai-Tibet Plateau, China.

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31 **Abstract**

32 1. Nitrogen's (N) retention is an important ecosystem function, particularly with
33 enriched soil nutrients due to global warming and anthropogenic activity. However,
34 theories and evidences of experiment as to how different plant community
35 components influence ecosystem N retention differ.

36 2. In this investigation, we constructed a ^{15}N label experiment to test how plant
37 community properties, including species richness, variances in dominance, functional
38 traits and diversity index, influence N uptake and retention. The three-year experiment
39 examined the effects of adding N and phosphorus (P) to an alpine meadow on the
40 Qinghai-Tibet Plateau.

41 3. Aboveground ^{15}N retention increased with the addition of N and P; by the end of
42 the experiment the addition of P recorded a significant increase of ^{15}N retention in
43 plants and soil. The change in soil nutrient conditions also facilitated a change in the
44 controls of ecosystem ^{15}N retention. Results for the control plots indicated that
45 ecosystem ^{15}N retention was controlled by greater species richness and root biomass;
46 plots with the addition of N showed larger community-weighted means (CWM) for
47 specific leaf area (SLA), and plots with additional P recorded lower CWM root
48 nitrogen contents (root N) and larger CWM root:shoot ratios (R/S).

49 4. *Synthesis.* Ecosystem ^{15}N retention was controlled by conservative and exploitative
50 plant species or their traits under N deficient and abundant conditions, respectively,
51 and under middle N conditions by species richness and community plant biomass.
52 Results from our investigation provide a potential universal rule for the controls of
53 ecosystem ^{15}N retention of natural alpine meadows in different succession stages
54 derived from soil N content. This finding increases our understanding of how different
55 plant community components influence ecosystem N retention.

56

57 **Key words:** nitrogen addition, phosphorus addition, ecosystem nitrogen retention,
58 species richness, functional traits, Alpine Meadow, the Qinghai-Tibet Plateau

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61 Introduction

62 Nitrogen (N) and phosphorous (P) nutrients are often limited in most terrestrial
63 ecosystems (Jing *et al.* 2016; LeBauer & Treseder 2008; Vitousek, Porder, Houlton, &
64 Chadwick 2010). However, due to increased mineralization of soil organic matter on
65 account of global warming (Rustad *et al.* 2001; Schmidt, Jonasson, Shaver, Michelsen,
66 & Nordin 2002) or anthropogenic activity (Falkowski *et al.* 2000; Vitousek *et al.*
67 1997), concentrations of nutrients in soils can increase. An increase in nutrient
68 availability frequently has multiple impacts on related ecosystem functioning and
69 services (Isbell *et al.* 2013; Jing *et al.* 2016; Smith, Tilman, & Nekola 1999),
70 indirectly by plant diversity loss (Hooper *et al.* 2012) and community structure
71 change (Klumpp & Soussana 2009; Stevens, Dise, Mountford, & Gowing 2004), but
72 the same due to minus effects on the soil systems (Bradley, Drijber, & Knops 2006).
73 As inorganic nitrogen can be readily lost by gas or leaching processes, ecosystem N
74 retention is a crucial grassland ecosystem role (De Vries & Bardgett 2016). In
75 terrestrial ecosystems, increasing evidence indicates that plant and soil systems are
76 expected to play vital roles in deciding N retention (Suding *et al.* 2008). Although soil
77 factors like pH, soil texture and content of soil organic matter are taken to involve
78 ecosystem N retention, the role plants play in N retention is largely unknown (De
79 Vries & Bardgett 2012), especially in natural communities.

80 Previous investigations have shown that species richness on ecosystem N retention
81 can have positive effects: plant trait measures can be affected due to raised contest for
82 resources and light (Roscher *et al.* 2012); plants can raise their N uptake due to an
83 increase in growth and aboveground biomass (Tilman, Wedin, & Knops 1996); plants
84 can lift their root biomass owing to below-ground over-yielding (Ravenek *et al.*
85 2014); or they can affect N retention directly by an increase in aboveground biomass
86 and evapotranspiration (Scherer-Lorenzen, Palmborg, Prinz, & Schulze 2003).
87 However, the mass-ratio hypothesis says that it is dominant plant species which
88 mastery ecosystem processes, not species richness (Grime 1998). For example, the
89 proportion of herbs in a community can independently affect community-weighted
90 mean (CWM) plant traits and the microbial community composition due to

91 species-specific impacts (Harrison & Bardgett 2010). These effects can likewise
92 influence plant ¹⁵N uptake and N leaching directly by affecting aboveground growth
93 and evapotranspiration (Craine *et al.* 2002). De Vries and Bardgett (2016) also found
94 that dominant plant traits control the destiny of short-term additional N in an
95 assembled plant and soil system.

96 Trait-based approaches have been increasingly used to fully understand the effects of
97 N retention on species diversity, individual species and functional group (Diaz *et al.*
98 2007; De Deyn, Cornelissen, & Bardgett 2008; Lavorel *et al.* 2013; Lavorel &
99 Garnier 2002). It has been ascertained that plant N uptake and N cycling processes
100 mediated by microbe were influenced by plant functional traits (Bardgett, Mommer,
101 & De Vries 2014; Grassein *et al.* 2015; Legay *et al.* 2014). Plant root biomass can
102 influence plant N retention by N and water uptakes (De Vries *et al.* 2012a), and the
103 microbe can be affected by providing resources (rhizodeposits) and competition for N
104 (Orwin *et al.* 2010). However, evidence for this is inconsistent. In addition, plant traits
105 have not fully explained plant N uptake (Moreau *et al.* 2015).

106 Therefore, there is currently a lack of consensus about the factors controlling
107 terrestrial ecosystem N retention or loss. Natural community properties for plant
108 species richness and the functional types of dominant species, as well as functional
109 traits for species and their community-level, were generally affected by abiotic factors
110 including temperature, light, water, nutrients availability, environmental
111 heterogeneousness and disturbance (Borer *et al.* 2014; Pausas & Austin 2001). From
112 these factors, nutrient availability is a recognized universal factor that drives
113 community structure and functions (LeBauer & Treseder 2008; Vitousek, Porder,
114 Houlton, & Chadwick 2010), partly as a result of the differences in resource
115 utilization between community species. For example, explorative plant species
116 generally have higher specific leaf area (SLA) and leaf N contents (leaf N) and lower
117 leaf dry matter content (LDMC) (Diaz *et al.* 2004; Wright *et al.* 2004) than
118 conservative plant species for certain root traits that have been recently certified
119 (Bardgett, Mommer, & De Vries 2014; De Vries & Bardgett 2016; Mommer &
120 Weemstra 2012). Under experimental conditions where additional nutrients were

121 added, community plant species richness generally decreased which resulted in a
122 change to the dominant species; plants with different resource uptake strategies
123 responded differently to the addition of nutrients. Explorative plant species generally
124 benefited due to their higher plasticity of functional response traits than conservative
125 plant species (Roderick, Berry, & Noble 2000). We speculate therefore that the control
126 factors on ecosystem N retention might change with changing soil nutrient
127 concentrations by changing plant growth, the dominant plant group or traits that are
128 associated with N retention.

129 In this investigation, a three-year ^{15}N label experiment was undertaken with research
130 plots receiving additional N and P nutrients to examine how plant community
131 properties influence ecosystem N retention under different nutrient availabilities. The
132 experiment was undertaken at the Haibei Alpine Meadow Ecosystem Research Station
133 (HBAMERS), an area which has been shown to be highly sensitive to anthropogenic
134 activities and global climate change (Piao *et al.* 2011; Shen *et al.* 2014; Yu, Luedeling,
135 & Xu 2010; Zhao 2009). The main focus of our investigation was species richness,
136 dominant leaf traits, trait functional diversity and divergence to contrast the species
137 diversity hypothesis, the mass-ratio hypothesis and the functional diversity hypothesis.
138 For the natural alpine meadow in HBAMERS, Liu *et al.* (2015) noted that primary
139 productivity was controlled by species richness; the addition of nutrients generally
140 increased the proportion of Gramineae biomass in the community which changed the
141 ecosystem function (Deng *et al.* 2014; Yang, Ren, Zhou, & He 2014). We
142 hypothesized that under control conditions, either species richness or the functional
143 trait diversity controls plant N uptake and ecosystem N retention through niche
144 complementarity and over-yielding. With the addition of N and P, dominant plant
145 proportion, explorative leaf traits will enhance N retention through greater plant N
146 uptake.

147

148 **Materials and methods**

149 **Experimental setup**

150 The experimental site was situated at HBAMERS (latitude 37°37' N and longitude
151 101°12' E). The station elevation is at 3200 m a.s.l, and the area has a typical plateau
152 continental climate with short, cool summers, and long, severely cold winters. Mean
153 annual temperature is 2 °C and mean annual precipitation is 500 mm; over 80% of
154 precipitation falls during the summer. The experiment was carried in an alpine
155 meadow dominated by *Kobresia humilis*, *Festuca sinensis*, *Elymus nutans*, *Poa*
156 *pratensis*, *Stipa purpurea*, *Carex tristachya*, *Gentiana straminea*, *Potentilla nivea* and
157 *Gentiana farreri*. Soil type is alpine meadow soil (Zhou and Wu, 2006) and the soil is
158 a Gelic Cambisol with an average thickness of 0.65 m (WRB, 1998).

159 A factorial N and P addition field experiment across 36 research plots (each plot
160 having an area of 3 m × 6 m) was undertaken in May, 2009. The addition of N and P
161 was undertaken using a randomized design. Nitrogen was added at a rate of 10 g N
162 m⁻² year⁻¹ (N addition) in the form of urea and phosphorus was added at a rate of 5 g P
163 m⁻² year⁻¹ (P addition) in the form of triple superphosphate. A control site was
164 established adjacent to each treatment plot to minimize the error of heterogeneity.
165 Nine repeat plots were established for each treatment and the control, respectively.
166 Typical nitrogen concentrations from the alpine grasslands of the Tibetan Plateau
167 (Jiang *et al.* 2013; Jing *et al.* 2016; Liu *et al.* 2012; Zheng *et al.* 2014) were used for
168 the nitrogen experiment. N and P were added once per year over a three-year period
169 (June 5th, 2009 to June 5th, 2011).

170 Each sample plot was divided into 12 micro-plots (March 12th, 2010) into which PVC
171 tubes (0.30 m diameter and 0.45 m high) were installed in soil. The micro-plots in
172 each main plot were divided into six ¹⁵N isotope labeling plots and six control plots.
173 The total number of micro-plots was 432. On the 20th and 21st July, 2013, isotope
174 labeling was carried out using ¹⁵NH₄¹⁵NO₃ (abundance 5%), and 38 mg ¹⁵N for each
175 micro-plot. In order to evenly distribute the ¹⁵N labeling in the micro-plots,
176 ¹⁵NH₄¹⁵NO₃ was dissolved in deionized water to make a 400 ml solution for each
177 micro-plot. 20 ml of ¹⁵NH₄¹⁵NO₃ solution was evenly injected (using a special
178 injector needle 35 mm long) into the top 0.3 m of the soil; this was repeated 20 times.
179 Before each injection, a 0.3 m deep hole was drilled using a steel drill. The syringe

180 was then immediately inserted to the bottom of the hole, and the solution was injected
181 at a constant speed whilst being elevated. Injection finished at the soil surface.
182 NH_4NO_3 was injected into the control micro-plot soils using the same method.

183 **Plant and soil sampling**

184 Plant and soil sampling was undertaken between the 25-27th August, 2011, coinciding
185 with the period of greatest biomass for the majority of the alpine meadow plants.
186 Plant families (species) sampled were: Gramineae (*E. nutans*, *P. pratensis*, *F. sinensis*,
187 *S. purpurea*, *Koeleria cristata*), Asteraceae (*Saussurea superb* and *Aster alpines*),
188 Cyperaceae (*K. humilis*, *K. pygmaea* and *C. tristachya*), Leguminosae (*Oxytropis*
189 *ochrocephala* and *Gueldenstaedtia verna*), Rosaceae (*Potentilla anserine* and *P. nivea*)
190 and Gentianaceae (*G. straminea* and *G. farreri*). Three plants were sampled per
191 species from the labeled micro-plots and the control micro-plots, respectively. Plant
192 shoots and roots were separated, rinsed with tap water and distilled water successively,
193 before being dried at 65 °C for 48h until a constant weight was recorded. Sample
194 weights were recorded before the samples were ground for N and ¹⁵N analyses.
195 Aboveground plant biomass was measured by clipping all living plants and collecting
196 litter from one micro-plot per plot. Each plant species was separated and weighed
197 after being oven-dried for 48 h at 65°C. In each micro-plot, soil moisture was
198 measured using Time-Domain Reflectometry (TDR) and three soil cores (3.5 cm
199 diameter) were extracted to investigate root biomass and soil properties in the top 0.3
200 m. Root samples were soaked in water and cleaned of residual soil using a 0.5-mm
201 sieve before being oven-dried at 65°C for at least 48 h. Root samples were weighed
202 and recorded as belowground biomass. Soil samples in each plot were aggregated
203 together, passed through a 2-mm sieve and stored in polyethylene bags before being
204 immediately transported to the laboratory where they were dried at 105 °C for 48h.
205 Soil pH values were determined using a combination glass electrode (soil:water W/V
206 ratio 1:2.5) .

207 **Trait analyses**

208 Shoots and roots of plants, aboveground vegetation and roots (for each micro-plot)
209 and soil were analyzed for N using an Elementar Vario EL elemental analyzer (Hanau,

210 Germany), and analyzed for ^{15}N using a spectrometer (DELTA V Advantage, Thermo
 211 Fisher Scientific, West Palm Beach, USA). Soil total P content was determined using
 212 a continuous flow auto-analyzer (Auto Analyzer III, Bran +Luebbe GmbH, Germany)
 213 after the samples were digested (340°C) with H_2SO_4 , using a mixture of K_2SO_4 and
 214 CuSO_4 as the catalyst.

215 ^{15}N excess atom% values, ^{15}N concentrations in samples, and total ecosystem ^{15}N
 216 retention values for each micro-plots were calculated using the following calculations:

217 $\text{atom\% excess } ^{15}\text{N} = \text{atom\% } ^{15}\text{N enriched} - \text{atom\% } ^{15}\text{N natural abundance};$

218 $^{15}\text{N sample (mg g}^{-1}) = \text{atom\% excess } ^{15}\text{N} \times \text{N sample (mg g}^{-1})/100;$

219 $^{15}\text{N pool} = ^{15}\text{N sample (mg g}^{-1}) \times \text{Dried weight of shoot, root or soil(g)};$

220 $^{15}\text{N retention (\%)} = (^{15}\text{N shoot pool} + ^{15}\text{N root pool} + ^{15}\text{N soil pool})$
 221 $(\text{mg}) \times 100 / 38(\text{mg}).$

222 Five healthy leaves were cut from five individuals per species per plot. Specific leaf
 223 area (SLA) was determined using an Epson flatbed scanner, and dried weight was
 224 determined using an electronic balance.

225 CWM for calculated leaf functional traits were estimated using species trait values
 226 and species relative abundance in the treatments, valued as dried weight (De Vries &
 227 Bardgett 2016; Garnier *et al.* 2004). Besides, trait functional diversity, trait functional
 228 divergence, functional richness, functional evenness, Rao's quadratic entropy
 229 (Mouchet, Vileger, Mason, & Mouillot 2010), evenness and Shannon's diversity were
 230 worked out using R software, as described by Laliberté and Shipley (2010).

231 **Statistical analyses**

232 Where appropriate, collected data was normality and log transformed. General linear
 233 models were used to test treatment effects on community and species-level trait
 234 measurements, N pools and retention. Species-level traits of root and leaf were tested
 235 using principal component analysis (PCA) and the R package. The traits correlations
 236 were analyzed using Spearman's rank correlations. Linear models were used to
 237 analyze the effects of species richness on plant community properties and plant leaf
 238 and root N content on ^{15}N uptake. All analyses were undertaken using R 3.2.0.

239 Indirect and direct controls of plant community properties, CWM traits, and trait
240 diversity and divergence on ecosystem N retentions and pools were tested using
241 structural equation modelling (AMOS 17.0). Based on our speculations and theoretic
242 knowledge of controls on plant N uptake and retention, the models were fitted by
243 selecting plant community properties according to their implication for interpreting
244 ^{15}N retentions in regression analyses. We modified model indices and removed
245 nonsignificant relationships stepwise, and these removals' impact on Akaike
246 information criterion (AIC) and model fit were tested by a likelihood ratio test. The
247 maximum likelihood χ^2 goodness-of-fit test, the root mean square error of
248 approximation (RMSEA), and the comparative fit index (CFI) were used to analyze
249 how well the models fitted the data (De Vries & Bardgett, 2016). The suitability of the
250 models was reflected by the test results: $P > 0.05$ for the non-significant χ^2 test; $P >$
251 0.05 for the high possibility of a low RMSEA value (Grace 2006; Pugesek, Tomer, &
252 von Eye 2003); and > 0.95 for a high CFI (Byrne 1994).

253

254 **Results**

255 **Treatment influences on plant community properties**

256 Results showed that soil total N ($P=0.003$, Table 1) and soil total P ($P < 0.001$, Table 1)
257 were affected by the treatments used in this investigation. Changes to soil moisture,
258 soil bulk density or soil pH due to the addition of N or P were not identified (Table 1).
259 The addition of N and P increased community aboveground biomass in the
260 micro-plots ($P < 0.001$, Table 2); the addition of N and P did not increase litter and
261 root biomass, or the ratio of shoots:roots.

262 Changes of species richness, evenness and Shannon's diversity were not significant
263 with the treatments, as well as the diversity index of functional diversity, functional
264 divergence, functional richness, functional evenness and Rao's quadratic entropy
265 (Table 3). CWM functional trait values for CWM leaf N, CWM root N and CWM
266 SLA values increased significantly with the addition of N and P (Table 3); the change
267 of CWM R/S was not significant.

268 On a community level, plants shoot and root $\delta^{15}\text{N}$ (‰), as $\delta^{15}\text{N}$ ratio of community
269 shoots and roots did not significantly change with the treatments (Table 4). Shoot N
270 content of the aboveground total community increased with the addition of both N
271 and P; the N ratio of community shoots and roots only significantly increased with the
272 addition of P (Table 4).

273 **Treatment influences on plant family-level measurements**

274 All of the aboveground biomass proportions for the seven plant families were not
275 affected by the treatments (Table S1). As a result of differences between leaf N and
276 root N for the plant families, and SLA and R/S (Table S2), leaf N and root N values
277 significantly increased under the addition of N and/or P for plants from Gramineae,
278 Asteraceae, Leguminosae and Rosaceae; leaf N and root N increased significantly for
279 Cyperaceae and Gentianaceae with an increase of N and P, respectively. Results for
280 SLA values showed a lack of consistency for changes among the plant families. For
281 Gramineae and Gentianaceae, SLA values increased and decreased with the addition
282 of N and P, respectively; they decreased with the addition of N for Asteraceae and
283 Leguminosae; and they increased with the addition of P for Leguminosae and
284 Rosaceae. R/S values decreased with the addition of P for Leguminosae and for
285 Gentianaceae with the addition of N.

286 Plant family-specific ^{15}N uptake varied strongly across the families (Table S3). Leaf
287 and root $\delta^{15}\text{N}$ values significantly decreased with the addition of P for Cyperaceae;
288 leaf $\delta^{15}\text{N}$ values significantly decreased with the addition of N and P, and root $\delta^{15}\text{N}$
289 values significantly decreased with the addition of N for Gentianaceae. Leaf $\delta^{15}\text{N}$
290 values significantly increased with the addition of N (Leguminosae) and N and P
291 (Rosaceae), respectively.

292 ^{15}N uptake efficiency significantly increased for Gramineae with an increase of N and
293 P, and for Rosaceae with the addition of P; results for Cyperaceae recorded a decrease
294 with the addition of P (Table 5). Significant changes were not recorded for the other
295 plant families with the addition of N or P.

296 **Influences of plant traits and community properties on ^{15}N retention**

297 PCA was more successful in separating leaf traits and R/S (Table S4), and the

298 correlations between these traits under different treatments varied (Table 6). Across all
299 plant families, leaf N was positively correlated with root N (control plots) and with
300 the addition of N and P. Root N was only negatively correlated with R/S with an
301 increase of P. SLA were positively correlated with leaf N, root N and R/S under the
302 control; there was only a positive correlation with root N with the addition of P and
303 there was no significant correlation with an increase in N.

304 Our results showed that species richness had significant effects on the aboveground
305 biomass of communities, the proportion of Gramineae in the plots, and two diversity
306 indices (evenness and Shannon's diversity; Table 7). Other community properties, such
307 as litter biomass, root biomass, total functional diversity indices and CWM of leaf and
308 root traits, could not be explained by species richness.

309 When the treatments of the control and N and P additions were analyzed together, no
310 effects of the aboveground N community on aboveground community ^{15}N uptake
311 ($y=96x+3100$, $R^2=-0.028$, $P=0.810$) were seen, or for the root N community on root
312 community ^{15}N uptake ($y=-399x+1216$, $R^2=0.028$, $P=0.166$). A trend of increasing
313 uptake of ^{15}N by the aboveground community with greater aboveground N under the
314 control (Fig. 1a), and decreased uptake of ^{15}N by the root community with greater root
315 N under N addition treatments (Fig. 1b) with independent regressions were identified.

316 Under different treatments, the amounts of ^{15}N retained in plant litter, roots and in the
317 soil were constant, but aboveground ^{15}N retention significantly increased with an
318 increase of N and P (Table 8). The total ^{15}N retention of the plant and soil system was
319 affected by the treatment, which significantly increased with the addition of P (Table
320 8).

321 Structural equation models (SEMs) showed that the amount of ^{15}N retained in the
322 plant and soil system was directly and indirectly controlled by plant traits and/or plant
323 community properties. SEMs for explaining ^{15}N retention under the control using
324 species and plant biomasses had a good fit (Chi-square=14.194, $df=14$, $P=0.435$;
325 comparative fit index=0.997; root mean square error of approximation=0.028,
326 $P=0.481$), and showed that the plant and soil system total ^{15}N retention was directly
327 controlled by ^{15}N retentions of aboveground, roots and soil. Aboveground biomass

328 directly controlled plant ^{15}N retentions, while species richness indirectly controlled
329 plant aboveground ^{15}N retentions through its effect on aboveground biomass. Species
330 richness directly controlled soil ^{15}N retention (Fig. 2a). Under the N addition
331 treatment, aboveground ^{15}N retention directly controlled the plant and soil system
332 total ^{15}N retention, which was indirectly controlled by CWM SLA by means of its
333 effect on aboveground biomass (Fig. 2b). This model's fit was good
334 (Chi-square=1.415, $df=3$, $P=0.702$; comparative fit index=1.000; root mean square
335 error of approximation<0.001, $P=0.708$). With the addition of P, the plant and soil
336 system total ^{15}N retention was directly controlled by root ^{15}N retention, and it was
337 indirectly controlled by both CWM root N and CWM R/S (Chi-square=1.669, $df=3$,
338 $P=0.637$; comparative fit index=1.000; root mean square error of
339 approximation<0.001, $P=0.645$) (Fig. 2c).

340

341 **Discussion**

342 On the community level, community aboveground biomass, CWM leaf and root N
343 contents, and CWM SLA significantly increased over the course of the N and P
344 addition experiment (2009-2011). Contrary to our expectation, species richness,
345 dominant plant species proportions and functional diversity indices were all not
346 affected. ^{15}N retention in the aboveground increased with the addition of N and P; the
347 treatment of adding P showed a significantly larger amount of ^{15}N retention in plants
348 and soil by the end of experiment. We hypothesized that under control conditions,
349 species richness or the functional trait diversity would increase ecosystem N retention
350 through niche complementarity and over-yielding; with the addition of N and P,
351 explorative plant proportion would enhance N retention by huger plant N uptake
352 through the changes of leaf traits. Therefore, our results back the hypothesis for the
353 addition of N and they partially confirm results for the control, however they do not
354 support the hypothesis for the addition of P.

355 In the control plot, total ^{15}N retention was jointly controlled by three components
356 (plant aboveground, root and soil ^{15}N retentions) and it was indirectly controlled by
357 plant species richness through controls on aboveground and soil ^{15}N retentions, and

358 by root biomass through controls on root ^{15}N retention. The over-yielding effect of
359 species richness was presented in aboveground biomass, but not in root biomass; this
360 was consistent with the findings of Tilman, Wedin, and Knops (1996). Greater
361 aboveground biomass has large evapotranspiration, which would result in raised water
362 uptake (Scherer-Lorenzen, Palmborg, Prinz, & Schulze 2003), both of which
363 increases plant ^{15}N uptake. An increase in root biomass also strongly increases the
364 total root N pool, and therefore ^{15}N retention (Fig. 2), thus improving root N uptake's
365 importance for ecosystem N retention (De Vries *et al.* 2012a; De Vries, Bracht
366 Jørgensen, Hedlund, & Bardgett 2015; Zogg, Zak, Pregitzer, & Burton 2000). Species
367 richness also affected soil ^{15}N retention, this was might from changes of soil microbes
368 and their activities, that were always, link to the plant species richness in natural
369 system (Landis, Gargas, & Givnish 2004; Liu *et al.* 2012; Wu, Hogetsu, Isobe, & Ishii
370 2007; Zak, Holmes, White, Peacock, & Tilman 2003).

371 In line with our expectations, aboveground biomass ^{15}N retention controls the
372 ecosystem ^{15}N retention in plots with an increase in N, this being indirectly increased
373 by CWM SLA through aboveground biomass. CWM SLA was the only trait involved
374 in our SEM to explain plant ^{15}N uptake. High CWM SLA characterized exploitative
375 growth strategies, indicating that exploitative plants can reduce the amount of ^{15}N
376 leached and increase ^{15}N retention in the plant and soil system. This finding was
377 corroborated by results from Grassein *et al.* (2015). In a soil community, bacteria and
378 fungi are easily affected by the condition of soil nutrients (Bloem, De Ruiter, &
379 Bouwman 1997; Van Veen & Paul 1979). In addition, exploitative traits, such as
380 greater CWM SLA, can indirectly increase soil microbial ^{15}N uptake by decreasing
381 the microbial C:N ratio, a character of more bacterial-dominated microbial
382 communities (De Vries *et al.* 2012b; Grigulis *et al.* 2013; Orwin *et al.* 2010). In this
383 study, although microbial ^{15}N uptake included in soil ^{15}N retention was not measured,
384 the influence of soil ^{15}N retention on the system total ^{15}N retention in the N addition
385 treatment, or for plant litter ^{15}N retention, was not identified; the quantity and quality
386 of plant litter has often been attributed to the link between plant traits and microbial
387 communities (Bardgett & Wardle 2010). In the N addition treatment, Gramineae

388 species were the major contributors to the large CWM SLA, though the proportion of
389 Gramineae biomass in the community had no significant control on CWM SLA in our
390 SEM (Table S2, Fig. 2). Therefore, it is more probable that aboveground plant actions
391 influenced community ^{15}N retention in the N addition treatment.

392 In contrast to our expectation, results for the P addition treatment showed that root ^{15}N
393 retention controls the ecosystem ^{15}N retention, which was directly decreased by CWM
394 root N and increased by CWM R/S. This result confirmed roots' important function of
395 in ecosystem N retention (De Vries *et al.* 2012a). Both lower CWM root N and higher
396 CWM R/S were characteristics of conservative growth strategies in the alpine
397 meadow on the Qinghai-Tibet Plateau (Zhou *et al.* 2016). This indicates that in these
398 conditions, conservative growth strategies can reduce the amount of ^{15}N leaching and
399 increase ^{15}N retention in the plant and soil system. These results were similar to
400 previous findings (De Vries *et al.* 2012a; De Vries & Bardgett 2012, 2016; Grigulis *et al.*
401 *et al.* 2013; Laughlin 2011).

402 In the three treatments, correlations identified between leaf and root traits give
403 support to previous investigations (Craine, Lee, Bond, Williams, & Johnson 2005; De
404 Vries & Bardgett 2016; Freschet, Cornelissen, van Logtestijn, & Aerts 2010; Roumet,
405 Urcelay, & Diaz 2006; Tjoelker, Craine, Wedin, Reich, & Tilman 2005), and they
406 assist the reality of a root economics spectrum (Table 6). However, explaining
407 ecosystem N retention using root traits must be viewed with caution. De Vries and
408 Bardgett (2016) showed that leaf traits were occasionally superior to root traits,
409 though root traits have been shown to have a firmer control on ecosystem N retention
410 and dynamics than aboveground functional traits (Bardgett, Mommer, & De Vries
411 2014; Grassein *et al.* 2015; Grigulis *et al.* 2013). In our investigation, functional traits
412 can explain ecosystem ^{15}N retention with the addition of N and P (Fig. 2), of which
413 the main competitive resources were light and soil N, and the importance of leaf trait
414 for SLA and root traits were evident in the treatments. So plant limited resources and
415 a lack of priority between leaf and root traits are the determinants explaining
416 ecosystem N retention and loss.

417 Collectively, our results show that the controls of ecosystem ^{15}N retention changed
418 with changing nutrient conditions in the soil. The species diversity hypothesis and the
419 mass-ratio hypothesis were supported in the control treatment and the mass-ratio
420 hypothesis was supported by an increase in N and P. Neither treatment supported the
421 functional diversity hypothesis. P addition treatment showed the lowest soil total N
422 among the treatments, which was derived from increasing soil N uptake by plants. In
423 fact, in terms of soil N content, our treatments formed a soil nitrogen gradient: P
424 addition treatment was a state of nitrogen deficiency; the control was in the middle
425 state; and the N addition treatment was a state of nitrogen abundance. Accordingly,
426 conservative plant traits, species richness and root biomass, and the exploitative plant
427 trait with increasing soil N availability controlled ecosystem ^{15}N retention.
428 Interestingly, this finding concurred with the characteristics of dominant plant species
429 in plant communities with different succession stages derived by soil N content.
430 Conservative plants and exploitative plants were the dominant species under soil N
431 deficient conditions and abundant condition, respectively, and the greatest species
432 richness generally existed in the middle soil N state in the alpine meadow (Zhao 2012;
433 Zhou, Yao, & Yu 2016). Thus, we can conclude that the controls of ecosystem ^{15}N
434 retention were determined by soil nutritional conditions for the natural alpine meadow
435 in different succession stages due to soil N content. This mechanism can provide
436 further understanding to how different plant community components influence
437 ecosystem N retention.

438

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446

447 **Author contributions**

448 B.Q.Y., W.Y.W., H.K.Z. and X.Q.Z. planned and designed the research; F.P.W, G.X.S.,
449 Z.M., and M.F.J. performed the experiment and analysed the data; and F.P.W and
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662 **Tables and figures**

663 **Table 1** Soil characters in the treatment plots. Values are mean \pm 1 SD. Different letters indicate
 664 significant differences ($P < 0.05$) among the treatments. Bold figures indicate P values < 0.05 .

Trait	Treatment			<i>P</i> -value
	Control	N addition	P addition	
Soil total N (%)	0.59 \pm 0.05b	0.56 \pm 0.03b	0.47 \pm 0.04a	<0.001
Soil total P (%)	0.052 \pm 0.002a	0.050 \pm 0.001a	0.081 \pm 0.005b	<0.001
Soil moist (%)	29.11 \pm 2.93	28.67 \pm 1.50	29.56 \pm 3.94	0.817
Soil bulk density (gcm ⁻³)	0.36 \pm 0.02	0.36 \pm 0.01	0.37 \pm 0.04	0.626
Soil pH (0-10cm)	7.50 \pm 0.13	7.47 \pm 0.11	7.42 \pm 0.08	0.182

665

666 **Table 2** Plant biomass (g per micro plot) and allocation between shoot and root under different
 667 treatments. Values are mean \pm 1 SD. Different letters indicate significant differences ($P < 0.05$)
 668 among the treatments. Bold figures indicate P values < 0.05 .

669

Trait	Treatment			<i>P</i> -value
	Control	N addition	P addition	
Aboveground biomass	19.20 \pm 5.72a	30.02 \pm 7.60b	26.02 \pm 5.41b	<0.001
Litter	20.61 \pm 7.89	19.74 \pm 3.18	19.98 \pm 2.62	0.929
Root biomass	183.94 \pm 94.15	160.50 \pm 34.04	154.45 \pm 38.71	0.544
Shoot and root ratio	0.25 \pm 0.09	0.32 \pm 0.08	0.31 \pm 0.07	0.098

670

671 **Table 3** Diversity index and CWM values under different treatments. Values are mean \pm 1 SD.
 672 Different letters indicate significant differences ($P < 0.05$) among the treatments. Bold figures
 673 indicate P values < 0.05 .

674

Diversity index	Treatment			<i>P</i> -value
	Control	N addition	P addition	
Functional diversity	0.85 \pm 0.34	1.05 \pm 0.27	0.84 \pm 0.24	0.240
Functional divergence	0.79 \pm 0.13	0.73 \pm 0.12	0.65 \pm 0.11	0.274
Functional richness	1.35 \pm 1.02	1.35 \pm 0.46	0.74 \pm 0.28	0.240
Functional evenness	0.52 \pm 0.13	0.54 \pm 0.11	0.49 \pm 0.09	0.965
Rao's quadratic entropy	1.38 \pm 0.66	1.79 \pm 0.56	1.37 \pm 0.44	0.206
CWM.Leaf N	1.84 \pm 0.15a	2.20 \pm 0.21b	2.20 \pm 0.17b	<0.001
CWM.Root N	1.83 \pm 0.15a	2.13 \pm 0.24b	2.27 \pm 0.12b	<0.001
CWM.R/S	1.00 \pm 0.37	1.08 \pm 0.23	0.83 \pm 0.18	0.217
CWM.SLA	65.31 \pm 9.58a	90.94 \pm 9.86c	79.64 \pm 9.95b	<0.001
Species richness	10.50 \pm 2.41	11.00 \pm 2.18	11.78 \pm 3.11	0.420
Evenness	0.51 \pm 0.12	0.58 \pm 0.08	0.47 \pm 0.09	0.107
Shannon's diversity	1.35 \pm 0.34	1.59 \pm 0.27	1.26 \pm 0.19	0.059

675 CWM, community-weighted mean; leaf N, leaf N content; root N, root N content; R/S, root shoot
 676 ratio; SLA, specific leaf area.

677

678 **Table 4** Values of community plant $\delta^{15}\text{N}$ (‰) and N (%) in shoots and roots. Values are mean \pm 1
 679 SD. Different letters indicate significant differences ($P < 0.05$) among the treatments. Bold figures
 680 indicate P values < 0.05 .
 681

Trait	Treatment			P-value
	Control	N addition	P addition	
Community shoot $\delta^{15}\text{N}$	3263 \pm 620	3536 \pm 883	3303 \pm 350	0.578
Community root $\delta^{15}\text{N}$	666 \pm 378	569 \pm 265	838 \pm 403	0.288
Community shoot N	2.38 \pm 0.17a	2.53 \pm 0.23ab	2.81 \pm 0.28b	<0.001
Community root N	1.30 \pm 0.24	1.43 \pm 0.15	1.29 \pm 0.21	0.259
$\delta^{15}\text{N}$ ratio of community shoot and root	6.03 \pm 2.72	7.14 \pm 2.95	4.67 \pm 1.99	0.152
N ratio of community shoot and root	1.90 \pm 0.45a	1.78 \pm 0.16a	2.28 \pm 0.31b	0.015

682
 683 **Table 5** ^{15}N uptake efficiency (mg ^{15}N /g dw. root) of plant families under the different treatments.
 684 Values are mean \pm 1 SD. Different letters indicate significant differences ($P < 0.05$) among the
 685 treatments. Bold figures indicate P values < 0.05 .
 686

Plant family	Treatment			P-value
	Control	N addition	P addition	
Gramineae	0.92 \pm 0.11a	1.14 \pm 0.22b	1.21 \pm 0.18b	0.001
Asteraceae	0.63 \pm 0.08	0.81 \pm 0.57	0.65 \pm 0.06	0.614
Cyperaceae	0.17 \pm 0.03b	0.18 \pm 0.05b	0.12 \pm 0.01a	0.006
Leguminosae	0.21 \pm 0.03	0.21 \pm 0.04	0.22 \pm 0.04	0.566
Rosaceae	0.80 \pm 0.34a	0.88 \pm 0.12a	1.42 \pm 0.08b	0.027
Gentianaceae	0.22 \pm 0.07	0.21 \pm 0.01	0.20 \pm 0.05	0.105

687
 688 **Table 6** Spearman's rank correlation of plant traits measured for all six plant families occurring in
 689 experiment treatments (n=216). Values indicate R values, *** indicate correlation is significant at
 690 the 0.001 level, ** at the 0.01 level and * at the 0.05 level.

		Leaf N	Root N	R/S	SLA
Control	Leaf N	1	0.43***	0.17	0.25*
	Root N		1	-0.08	0.33***
	R/S			1	0.26**
	SLA				1
N addition	Leaf N	1	0.62***	0.07	-0.02
	Root N		1	-0.15	0.05
	R/S			1	-0.08
	SLA				1
P addition	Leaf N	1	0.74***	-0.09	0.10
	Root N		1	-0.28*	0.42**
	R/S			1	0.10
	SLA				1

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692 **Table 7** Statistics for linear models of species richness effects on plant community properties.
 693 Bold figures indicate P values < 0.05.
 694

Predictor	Response variable	R^2	F-value	P-value
Species richness	Aboveground biomass	0.098	4.819	0.035
	Litter	0.014	1.507	0.228
	Root biomass	-0.022	0.246	0.623
	Gramineae proportion	0.127	6.083	0.019
	Functional diversity	-0.022	0.245	0.624
	Functional divergence	0.037	2.355	0.134
	Functional richness	0.014	1.509	0.228
	Functional evenness	0.024	1.845	0.183
	Rao's quadratic entropy	-0.026	0.120	0.731
	CWM.Leaf N%	-0.023	0.224	0.639
	CWM.Root N%	0.009	1.305	0.261
	CWM.R/S	-0.025	0.154	0.697
	CWM.SLA	-0.014	0.519	0.476
	Evenness	0.129	6.165	0.018
	Shannon's diversity	0.097	4.766	0.036

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697 **Table 8** Community ^{15}N retention properties under the different treatments. Different letters
 698 indicate significant differences ($P < 0.05$) among the treatments. Bold figures indicate P values <
 699 0.05.
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Trait	Treatment			P -value
	Control	N addition	P addition	
^{15}N retention in aboveground (mg)	5.06±1.32a	8.63±3.40b	7.58±2.52b	0.001
^{15}N retention in litter (mg)	1.51±0.81	1.48±1.03	2.22±1.08	0.155
^{15}N retention in root (mg)	4.08±2.21	4.13±1.97	5.31±3.34	0.455
Total ^{15}N retention in plant (mg)	10.65±2.59a	14.24±5.02ab	15.11±3.35b	0.006
Shoot and root ^{15}N retention ratio	2.11±1.29	2.76±0.95	2.66±1.75	0.417
^{15}N retention in soil (0-30cm) (mg)	10.59±2.00	10.73±2.22	10.84±2.52	0.962
^{15}N retention (%)	55.90±7.85a	65.71±16.00ab	68.27±10.13b	0.015

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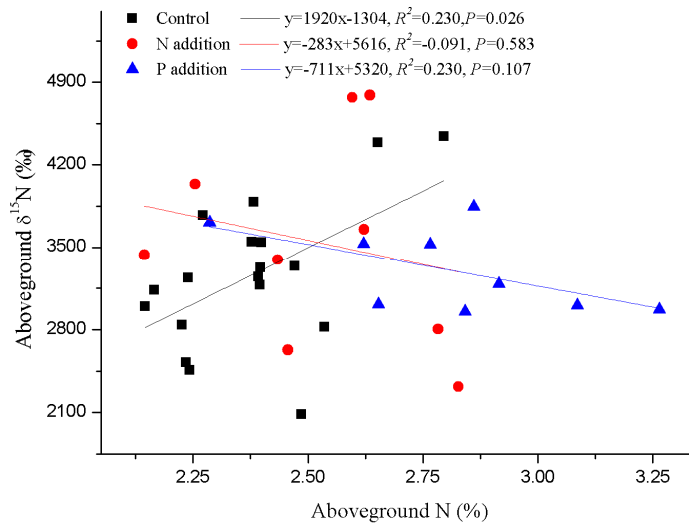
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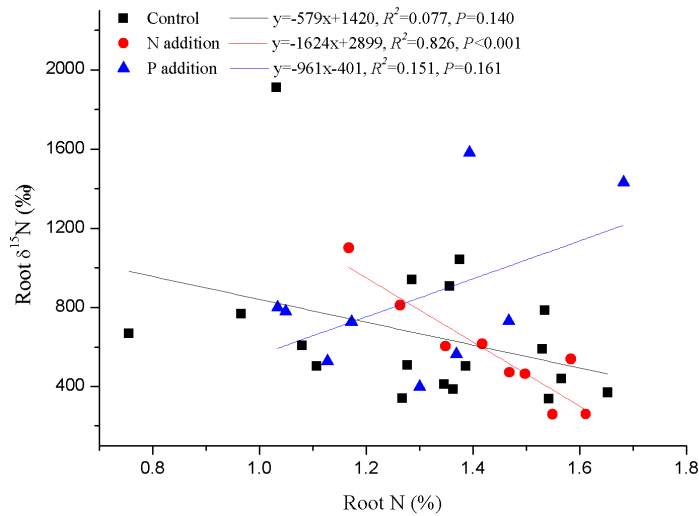
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Figure 1 Specific ^{15}N uptake as explained by community level trait aboveground N content (a) and root N content (b) for the different treatments.

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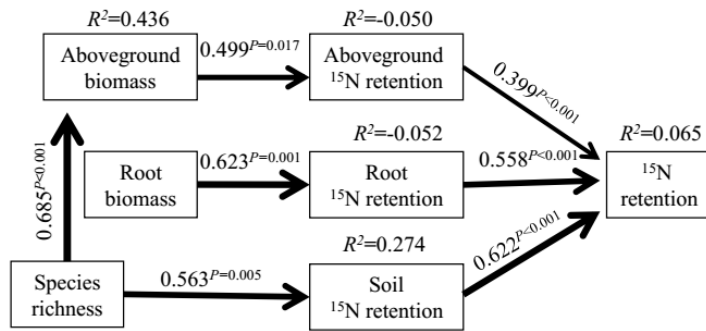
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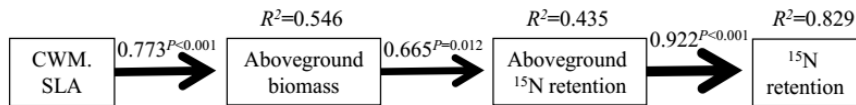
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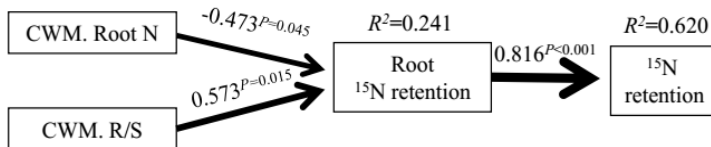
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736 **Figure 2** Most parsimonious models for explaining ecosystem ^{15}N retention in the
 737 control (a) (Chi-square=14.194, df=14, $P=0.435$; comparative fit index =0.997; root
 738 mean square error of approximation=0.028, $P=0.481$), N addition (b)
 739 (Chi-square=1.415, df=3, $P=0.702$; comparative fit index =1.000; root mean square
 740 error of approximation<0.001, $P=0.708$) and P addition (c) (Chi-square=1.669, df=3,
 741 $P=0.637$; comparative fit index=1.000; root mean square error of
 742 approximation<0.001, $P=0.645$), using species richness, plant biomass and
 743 community-weighted mean (CWM) leaf and root traits. The weight of the arrows
 744 indicates the strength of the causal relationship, supplemented by a standardized path
 745 coefficient and P -value. R^2 values denote the amount of variance explained by the
 746 model for the response variables. SLA, specific leaf area.

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758 **Supporting Information**

759 **Table S1** Aboveground biomass proportions of plant family biomass* (exclusive of litter) under
 760 different treatments, Values are mean \pm 1 SD.

Plant family	Treatment			P -value
	Control	N addition	P addition	
Gramineae	0.68 \pm 0.10	0.63 \pm 0.08	0.72 \pm 0.07	0.111
Asteraceae	0.05 \pm 0.06	0.08 \pm 0.02	0.04 \pm 0.02	0.183
Cyperaceae	0.03 \pm 0.03	0.04 \pm 0.01	0.01 \pm 0.00	0.056
Leguminosae	0.07 \pm 0.05	0.10 \pm 0.07	0.10 \pm 0.05	0.220
Rosaceae	0.06 \pm 0.05	0.07 \pm 0.02	0.04 \pm 0.01	0.220
Gentianaceae	0.06 \pm 0.04	0.03 \pm 0.02	0.03 \pm 0.01	0.070
Others	0.06 \pm 0.05	0.06 \pm 0.02	0.06 \pm 0.03	0.982

761 * in which Gramineae include *Elymus nutans*, *Poa pratensis*, *Festuca sinensis*, *Stipa purpurea*,
 762 *Koeleria cristata*; Asteraceae include *Saussurea superb* and *Aster alpines*; Cyperaceae include
 763 *Kobresia humilis*, *K. pygmaea* and *Carex tristachya*; Leguminosae include *Oxytropis*
 764 *ochrocephala* and *Gueldenstaedtia verna*, Rosaceae include *Potentilla anserine* and *P. nivea*; and
 765 Gentianaceae include *Gentiana straminea* and *Gentiana farreri*.

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767 **Table S2** Leaf and root trait values per plant family under the different treatments. Values are
 768 mean \pm 1 SD. Different letters indicate significant differences ($P < 0.05$) among the treatments.
 769 Bold figures indicate P values < 0.05 .

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Plant	Trait	Treatment			P -value
		Control	N addition	P addition	
Gramineae	Leaf N (%)	1.76 \pm 0.17a	2.14 \pm 0.31b	2.09 \pm 0.20b	<0.001
	SLA(mm ² /mg)	56.54 \pm 9.33a	97.81 \pm 9.92c	71.39 \pm 14.59b	<0.001
	Root N (%)	1.86 \pm 0.18a	2.10 \pm 0.36b	2.27 \pm 0.14b	0.001
	R/S	0.53 \pm 0.13	0.59 \pm 0.20	0.44 \pm 0.09	0.085
Asteraceae	Leaf N (%)	1.87 \pm 0.23a	1.94 \pm 0.19ab	2.12 \pm 0.12b	0.003
	SLA(mm ² /mg)	150.67 \pm 40.70b	115.45 \pm 27.25a	146.67 \pm 8.54ab	0.028
	Root N (%)	2.06 \pm 0.21a	2.31 \pm 0.49ab	2.43 \pm 0.38b	0.038
	R/S	1.07 \pm 0.53	0.85 \pm 0.32	0.76 \pm 0.35	0.194
Cyperaceae	Leaf N (%)	1.52 \pm 0.22a	1.70 \pm 0.28ab	1.90 \pm 0.12b	0.002
	SLA(mm ² /mg)	54.57 \pm 9.40	63.33 \pm 17.07	67.19 \pm 11.41	0.064
	Root N (%)	1.45 \pm 0.21	1.54 \pm 0.10	1.48 \pm 0.07	0.555
	R/S	3.62 \pm 0.91	3.62 \pm 1.66	3.65 \pm 0.83	0.995
Leguminosae	Leaf N (%)	2.85 \pm 0.30a	3.20 \pm 0.21b	3.07 \pm 0.19ab	0.002
	SLA(mm ² /mg)	101.34 \pm 8.53b	84.94 \pm 6.00a	119.69 \pm 3.87c	<0.001
	Root N (%)	2.33 \pm 0.31a	2.48 \pm 0.29ab	2.67 \pm 0.11b	0.004
	R/S	3.97 \pm 1.08b	4.11 \pm 1.47b	2.75 \pm 0.63a	0.021
Rosaceae	Leaf N (%)	1.98 \pm 0.18a	2.10 \pm 0.15a	2.50 \pm 0.05b	<0.001
	SLA(mm ² /mg)	12.47 \pm 1.74a	19.58 \pm 5.34a	51.17 \pm 20.51b	<0.001
	Root N (%)	1.57 \pm 0.23a	2.00 \pm 0.22b	2.24 \pm 0.16c	<0.001
	R/S	0.64 \pm 0.29	0.72 \pm 0.36	0.58 \pm 0.16	0.586

Gentianaceae	Leaf N (%)	2.02±0.04	1.94±0.12	1.91±0.41	0.472
	SLA(mm ² /mg)	108.98±23.91b	90.04±9.28a	91.71±7.49a	0.001
	Root N (%)	1.40±0.03b	1.28±0.06a	1.43±0.07b	<0.001
	R/S	2.42±0.80b	1.31±0.56a	2.33±0.33b	0.001

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772 **Table S3** Leaf and root $\delta^{15}\text{N}$ (‰) values per plant family under the different treatments. Values are
 773 mean \pm 1 SD. Different letters indicate significant differences ($P < 0.05$) among the treatments.
 774 Bold figures indicate P values < 0.05 .

775

Plant family	Trait	Treatment			P -value
		Control	N addition	P addition	
Gramineae	Leaf	4105±499	4462±1215	3847±339	0.224
	Root	2963±673	2948±1015	2866±489	0.955
Asteraceae	Leaf	3353±702	3938±1916	2676±300	0.070
	Root	2747±776	3236±1976	2062±339	0.117
Cyperaceae	Leaf	2937±703b	2627±533b	1158±144a	<0.001
	Root	934±409b	771±614ab	410±87a	0.016
Leguminosae	Leaf	806±362a	1000±174b	566±222a	0.001
	Root	485±198	477±172	351±117	0.143
Rosaceae	Leaf	3343±737a	4201±357b	4455±239b	<0.001
	Root	3325±725	3134±329	2769±38	0.108
Gentianaceae	Leaf	2168±318b	1637±76a	1672±554a	0.001
	Root	1386±280b	1012±96a	1150±219ab	0.002

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777 **Table S4** Component Matrix of Principal Component Analysis for leaf and root traits.

	Component	
	1	2
CWM.Leaf N	0.881	0.189
CWM.Root N	0.913	-0.035
CWM.R/S	-0.088	0.990
CWM.SLA	0.781	-0.060

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