Greater impacts of reduced than oxidized nitrogen on grassland plant species losses

Running title: Impacts of nitrogen forms on plant richness

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Junyi Liang designed the study. Suxian Ren and Xun Sun conducted the meta-analysis. Suxian Ren conducted the greenhouse experiment and analyzed data with assistant of Tianci Huo, Xiaowei Gou and Ru Hou. Suxian Ren and Junyi Liang wrote the early drafts of the manuscript. All authors contributed to the revision and approved the final manuscript.

Data availability statement

The data and R code are available in figshare at

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Abstract

Increasing atmospheric nitrogen (N) deposition is a major threat to terrestrial vegetation biodiversity. The reactive N deposition includes reduced (NH_x) and oxidized (NO_y) forms, which play different roles in a variety of biological processes. Whether NH_x and NO_y affect the vegetation biodiversity differently or equivalently has been paid little attention despite decades of research on biodiversity changes in response to N deposition. Combining a meta-analysis and an N gradient experiment, we found that reduced N (i.e., NH_4^+) addition resulted in a significant species loss of forbs. By contrast, oxidized N (NO_3^-) addition showed a much weaker impact on species richness. The greater impact of reduced than oxidized N on the species loss could be due to the susceptibility of forbs to NH_4^+ toxicity and nitrification-induced acidification, which weakened their competition for light and other resources compared with grasses. These results provide direct evidence that reduced N exerts greater impacts on grassland plant species losses than oxidated N, emphasizing that the ratio of NH_x to NO_y, in addition to the total N deposition amount, should be considered as an important predictor of grassland biodiversity losses.

Key words: nitrogen deposition; vegetation biodiversity; reduced/oxidized nitrogen ratio; ammonium toxicity; acidification

INTRODUCTION

The amount of reactive nitrogen (N) deposition to terrestrial ecosystems has doubled since 1970 due to increased industrial and agricultural emissions (Galloway *et al.* 2004; Galloway *et al.* 2008; Reay *et al.* 2008; Fowler *et al.* 2013). Increased N deposition, which has profound effects on ecosystem productivity and diversity, generally stimulates the productivity of terrestrial ecosystems at the cost of biodiversity loss (LeBauer & Treseder 2008; Hautier *et al.* 2015; Borer & Stevens 2022; Stevens *et al*. 2018). It has widely been observed that long-term N addition can reduce plant species richness and result in local species extinction (Stevens *et al.* 2004; Isbell *et al.* 2013; Storkey *et al.* 2015; Tian *et al.* 2016; Xiao *et al.* 2020). A major mechanism of biodiversity loss by N deposition is the asymmetric nature of competition for light (Hautier *et al.* 2009; Eskelinen *et al.* 2022). Nitrogen addition disproportionally stimulates the growth of taller plants that have better access to light, which further suppresses the light access and growth of shorter plants (Holt *et al.* 1994; Borer *et al.* 2014). However, fertilization can reduce plant biodiversity even with light addition in the understory (Eskelinen *et al.* 2022), suggesting that other mechanisms are also vital in affecting plant biodiversity. For example, soil acidification, ammonium toxicity and metal toxicity, which can suppress vegetative and reproductive growths, may lead to species losses (Stevens *et al.* 2006; Stevens *et al.* 2010; Basto *et al.* 2015; Tian *et al.* 2016; Zheng *et al.* 2019).

However, not all forms of N addition can result in soil acidification, ammonium toxicity and metal toxicity. These toxic processes are primarily related to reduced

76   instead of oxidized N (Esteban *et al.* 2016). Ammonium nitrogen (NH_4^+) is usually

77   nitrified to NO_3^- rapidly, accompanied by H^+ release (Muller *et al.* 2006; Stevens *et al.*

78   2011). When plants absorb NH_4^+, H^+ is overflowed into the soil to maintain the

79   intracellular charge balance (Britto & Kronzucker 2002). As a result, nitrification and

80   NH_4^+ absorption acidify the soil. In addition, plants would suffer from NH_4^+ and

81   metal toxicities under NH_4^+ excess conditions, showing inhibited growth and even

82   death (Britto & Kronzucker 2002; van den Berg *et al.* 2008; Hachiya *et al.* 2021).

83   Therefore, it is fairly reasonable to hypothesize that reduced N may exert a greater

84   impact than oxidized N on plant biodiversity loss. However, studies that focus on the

85   impact of N on biodiversity usually emphasize the importance of N deposition

86   dosage, but cannot reveal the possible impacts of different N forms (Clark & Tilman

87   2008; Hautier *et al.* 2015; Midolo *et al.* 2019; Xiao *et al.* 2021). To date, direct

88   evidence is still lacking whether reduced and oxidized N have different impacts on

89   biodiversity.

90   Given the fact that the ratio of NH_x to NO_y deposition keeps changing under

91   global change (Clark & Tilman 2008; Reay *et al.* 2008; Li *et al.* 2016; Ackerman *et*

92   *al.* 2019; Yu *et al.* 2019), it is vital to understand how different forms of N deposition

93   affect plant biodiversity. In this study, we first performed a meta-analysis to reveal the

94   global pattern of grassland plant biodiversity in response to N addition. Then, we

95   conducted an experiment that manipulates N addition forms and levels to test whether

96   both reduced and oxidated N can mimic the global pattern. After that, we explored the

97   different mechanisms of reduced and oxidated N in affecting grassland plant

98   biodiversity.

99

100   **MATERIALS AND METHODS**

101   **Meta-analysis**

102   We searched peer-reviewed publications from 1990 to 2022 using the Web of

103   Science. The search keywords were ("nitrogen deposition" or "nitrogen application"

104   or "nitrogen addition" or "nitrogen enrichment" or "nitrogen input" or "nitrogen

105   fertilization" or "nitrogen treatment") and ("plant richness" or "plant diversity" or

106   "plant evenness" or "plant composition" or "plant number") and ("grassland" or

107   "meadow" or "steppe" or "prairie"). Studies were chosen for the meta-analysis based

108   on the following criteria: (1) the means, standard deviations (SD) or standard errors

109   (SE), and sample sizes of the variables were provided; (2) at least one plant

110   community index –richness, Shannon-Wiener diversity, Simpson diversity or

111   evenness – was reported or could be calculated; (3) if more than one publication

112   presented results from an identical experiment, only the latest one was chosen; and (4)

113   only field experiments were selected. Overall, 51 studies were included in the meta-

114   analysis (TableS1, Figure S1).

115   We used the log-transformed response ratio – ln(RR) – by following Hedges *et*

116   *al.* (1999) to assess the effect size of N addition:

117    $$\ln(RR) = \ln(X_t/X_c)$$

118   where X_t and X_c are the mean values of the variable in the N addition treatment and

119   the control, respectively.

The variance (v) of ln(RR) was calculated as:

$$v = \frac{S_t{}^2}{n_t X_t{}^2} + \frac{S_c{}^2}{n_c X_c{}^2}$$

where S_t and S_c are the standard deviations, n_t and n_c are the sample sizes for the treatment and control groups, respectively. The weighting factor (w_{ij}) was calculated as:

$$w_{ij} = \frac{1}{v}$$

The weighted response ratio (RR$_{++}$) was calculated as:

$$RR_{++} = \frac{\sum_{i=1}^{m} \sum_{j=1}^{ki} w_{ij} RR_{ij}}{\sum_{i=1}^{m} \sum_{j=1}^{ki} w_{ij}}$$

We tested publication bias with a funnel plot, and the observed pattern indicated no sign of publication bias (Figure S2). We used the R package "metafor" (Viechtbauer 2010) to calculate the 95% confidence intervals (CIs). When the 95% CIs did not overlap with zero, the effect of N addition was considered significant. The linear regression was used to examine the relationships between ln(RR) of species diversity and the N addition levels.

Greenhouse experiments

Experiment design

In order to explore responses of different plant functional groups to N addition, we selected four widespread species in China's temperate grasslands, including *Leymus chinensis* (Grass), *Astragalus laxmannii* (Legume), *Carex korshinskyi* (Sedge) and *Potentilla chinensis* (Forb). In October 2021, we tested the germination rate (g_i)

141 and emergence time of the four plant seeds. Then, we sowed seeds in pots (10 cm

142 width, 10 cm height) filled with quartz sand and vermiculite according to the

143 emergence time to ensure the emergence date of the four plants was as close as

144 possible. Two experiments – monocultural and mixed systems – were set

145 simultaneously. The seeding amount of the monoculture was $1/g_i \times 20 \times 3$ seeds/pot,

146 and that of mixed cropping was $1/g_i \times 5 \times 3$ seeds/per plants/pot. In total, we set up 24

147 pots of the mixed system, and 96 pots of monocultural plants (Fig S3). We configured

148 a nutrient solution without N (i.e., Hoagland solution with N removed), and applied

149 an identical amount of nutrient solution to each pot every day to ensure plant growth.

150 After 10 days of sowing, plants in each pot were thinned to 20 individuals in the

151 monocultural pots, and 5 plants were reserved for each species in the mixed pots. The

152 pots were randomly divided into two groups, with half receiving reduced N

153 $((NH_4)_2SO_4)$ and the other half receiving oxidized N $(Ca(NO_3)_2)$ every three days for

154 thirty days, eventually reaching an N fertilization level of 25, 50, 100, and 200 mg N

155 pot^{-1}. After that, the Hoagland solution was provided to all pots until harvesting.

156 We harvested the plants on the 80th day after thinning. Then, plant height, leaf

157 area, leaf C/N ratio, root traits in the monocultural pots, and plant aboveground

158 biomass in both monocultural and mixed-cultural systems were measured.

159 Immediately after the harvesting, plant leaves were spread and scanned in greyscale at

160 the resolution of 300 dpi (Canon Co., Ltd., model Canon ScanLIDE300). The total

161 leaf area (LA, cm^2) was calculated with Image J V1.41 (Wayne Rasband, 2008). Then,

162 all the leaves were oven-dried at 75 °C for 48 h and weighed. The nitrogen

163 concentration and carbon concentration were determined using an elemental analyzer

164 (Vario Macro, element, Germany). The weights of stems and leaves were summed to

165 represent the aboveground biomass (AGB). Specific leaf area (SLA) was calculated as

166 the total leaf area divided by its leaf's dry matter. Root samples were flushed with

167 running water, spread out on a plastic plate and scanned in greyscale at the resolution

168 of 600 dpi (Perfection V750 Pro, Epson, Suwa, Japan). These scanned images were

169 analyzed using WinRHIZO (Regent Instruments, Quebec, Canada) to derive total root

170 length, volume, surface area, diameter (Dm) and fine root (diameter < 0.2mm) length.

171 Then, they were oven-dried at 75 °C for 48 h and weighed as belowground biomass

172 (BGB). Specific root length (SRL) was calculated as the total root length divided by

173 its dried mass. Root tissue density (RTD) was calculated as the root dry mass divided

174 by its volume. Fine root proportion (FRP) was calculated as the fine root length

175 divided by its total root length. The root-to-shoot ratio (RSR) was calculated as the

176 belowground biomass over its aboveground biomass.

177

178 Statistical analyses

179   Linear mixed-effects models (LMMs) were constructed with N forms and levels

180 as fixed factors and the replication as a random factor to assess the effect of N forms

181 and levels on the total plant numbers, AGB, Shannon-Wiener diversity and Simpson

182 diversity under the mixed cultural systems. In addition, the multiple comparison test

183 (Tukey's test) was adopted to explore the changes in total plant numbers and AGB

184 with N levels under each N form. To assess the effect of different species, N forms

185   and N levels on AGB and plant functional traits (PFTs, including BGB, Height, Leaf

186   area, SLA, C/N, Leaf N, SRL, RSR, RTD, FRP, Dm) in the monocultural system,

187   LMMs were employed with species, N forms and levels as fixed factors and

188   replication as a random factor. To explore the differences in AGB and PFTs of each

189   species under different treatments, LMMs with N forms and levels as fixed factors

190   and replication as a random factor were used. The Pearson Correlation method was

191   used to explore the relationship between PFTs and AGBs of different species under

192   the two N forms. Additionally, we also investigated the differences of PFTs among

193   different species and the trade-off relationship of PFTs through principal component

194   analysis (PCA) using the packages "FactoMineR" and "factoextra". All analyses were

195   performed in R4.0.2 (R Core Team, 2020).

196

197   **RESULTS**

198   **The meta-analysis of effects of N addition on grassland biodiversity**

199   Across the global grasslands, 90% (46/51) of studies used NH_4NO_3 or urea as the

200   N treatment (Table S1). Plant species richness, Shannon-Wiener diversity and

201   evenness were reduced by N addition (Fig. 1a). Specifically, the reduced plant species

202   richness was primarily due to the loss of non-grass species (Fig. 1b). In addition, the

203   decreases in grassland biodiversity were strongly dependent on the N-addition levels

204   ($P < 0.001$), showing greater responses of plant species richness, Shannon-Wiener

205   diversity and evenness to higher levels of N addition (Fig. 1c-e).

206

Effect of N addition on the mixed cultural system

The total number of plants, AGB, Shannon-Wiener diversity and Simpson diversity were significantly affected by N forms, N levels and their interactions (Table1, $P < 0.05$). The total plant number decreased along the gradient of NH_4^+-N application (Fig. 2a). Specifically, *Astragalus laxmannii* and *Potentilla chinensis* disappeared at the highest rate of NH_4^+-N application (i.e., 200mg pot^{-1}). The effect of NO_3^--N application was milder, showing no significant changes in the plant number at low rates (< 100mg pot^{-1}; $P > 0.05$; Fig. 2b). At the highest rate of NO_3^--N application, the plant number was reduced but all the four species presented. Moreover, the Shannon-Wiener diversity and Simpson diversity decreased with the level of NH_4^+-N, while showing a neutral pattern with the level of NO_3^--N application (Fig. S4a, b). Total aboveground biomass of the mixed cultural system was significantly increased by the application of NO_3^--N, but not by the addition of NH_4^+-N (Fig. 2c, d). *Leymus chinensis* contributed the most to total AGB. Moreover, the aboveground biomass of *Astragalus laxmannii* and *Potentilla chinensis* were 0 due to local extinction under the highest-level NH_4^+-N application.

Response of different species to N addition in the monocultural system

All PFTs were significantly discrepant in different species (Table S2, $P < 0.001$). The effect of NO_3^--N treatments were higher than NH_4^+-N treatments on AGB ($P < 0.01$; Table 2, Fig. 3). The aboveground biomass of *Leymus chinensis* increased with the level of NO_3^--N application, but was not changed significantly by NH_4^+-N (Fig.

3a). The application of NO_3^--N increased aboveground biomss of *Astragalus*

laxmannii and *Potentilla chinensis*, while NH_4^+-N application inhibited their AGB

(Fig. 3b, d).

Functional traits of the four species changed differently with N addition (Fig. S5-

S8). The plant height, LA and BGB of all species were higher under NO_3^--N than

NH_4^+-N treatments (Fig. S5-S8). For all four species, leaf N concentration increased,

but RSR decreased, with the N addition level (S5-S9). The aboveground biomass of

Leymus chinensis and *Carex korshinskyi* were positive correlated with leaf N

concentration but negative correlated with leaf C/N and RSR. The correlations of

AGB with leaf N concentration, leaf C/N ratio and RSR were weaker under NH_4^+-N

than NO_3^--N treatments (Fig. 4, S10). In addition, the PCA discriminated PFTs of the

four species, showing that *Leymus chinensis* had greater AGB, leaf area, height, SRL,

BGB, FRP and Dm than other species, regardless NO_3^--N or NH_4^+-N applications

(Fig. 5).

DISCUSSION

Different effects of reduced and oxidized N on grassland biodiversity

The meta-analysis showed that the reduced grassland biodiversity caused by N

addition is mainly due to the loss of non-grass species, indicating that the competitive

edge of grasses over non-grass species was enhanced by N addition (Hautier *et al.*

2009; DeMalach *et al.* 2017). Higher N additions were associated with greater

biodiversity reductions, suggesting that N limitation favors species coexistence, while

251   negative inter-species interactions lead to the suppression of vulnerable species when

252   N is enriched (Callaway *et al.* 1991; Wang *et al.* 2013; Borer *et al.* 2014). Among the

253   51 collected studies, 46 (i.e., 90%) studies used NH_4NO_3 or urea as the N treatment.

254   Given the rapid transformations from urea to ammonium and from ammonium to

255   nitrate in the soil (Stevens *et al.* 2011), the treatments of NH_4NO_3 and urea usually

256   mix the effect of reduced and oxidized N. As a result, the meta-analysis and collected

257   studies could not compare different N forms in influencing the grassland biodiversity

258   loss.

259   The species loss was reproduced in our mixed cultural system with NH_4^+-N

260   treatments but not with NO_3^--N treatments. The number of non-grass species in the

261   mixed cultural system decreased with the application level of NH_4^+-N application.

262   The highest concentration of NH_4^+-N application (i.e., 200 mg N pot^{-1}) led to the loss

263   of *Astragalus laxmannii* and *Potentilla chinensis*. By contrast, although the severest

264   NO_3^--N treatment reduced the number of *Astragalus laxmannii* and *Potentilla*

265   *chinensis*, they were not extinct. Meanwhile, the productivity of the mixed cultures

266   increased continuously along the NO_3^--N level, but was not changed along the NH_4^+-

267   N gradient (Fig. 2). These results indicate that the impact of NO_3^--N application on

268   plant biodiversity is primarily related to the asymmetric growths of grasses and non-

269   grass species, supporting the mechanism of light competition between tall and short

270   species (Hautier *et al.* 2009; Xiao *et al.* 2021; Eskelinen *et al.* 2022). However, NH_4^+-

271   N affected plant biodiversity more drastically, suggesting that NH_4^+-induced

272   physiological changes may play a critical role in affecting the species loss, which was

273 verified by our monocultural experiment. In the monocultural system, the

274 aboveground biomass of *Leymus chinensis* and *Carex korshinskyi* increased with NO_3^-

275 -N levels, but showed neutral responses to NH_4^+-N treatments. Moreover, the growths

276 of *Astragalus laxmannii* and *Potentilla chinensis* were significantly suppressed by

277 NH_4^+-N, which are consistent with our hypothesis that even without inter-species

278 interactions, NH_4^+-N-related physiological toxicity is vital in affecting the non-grass

279 species loss. The results are supported by previous studies that have shown significant

280 susceptibilities of forbs to soil acidification (Stevens *et al.* 2010; Ward *et al.* 2017;

281 Kimmel *et al.* 2020; Xiao *et al.* 2020), ammonium toxicity (Britto & Kronzucker

282 2002; Esteban *et al.* 2016), and manganese ions toxicity (Horswill *et al.* 2008; Tian *et*

283 *al.* 2016).

284

285 **Responses of plant functional traits to reduced and oxidized N**

286   Functional traits can determine plant responses to resource enrichment by

287 influencing plant competitiveness and environmental tolerance (Diaz & Cabido 2001;

288 Reich *et al.* 2003; Bardgett *et al.* 2014). The aboveground biomass, plant height, leaf

289 area and BGB of all species were higher under NO_3^--N treatments than NH_4^+-N

290 treatments, indicating that NO_3^--N treatments favor plant growth. The four species

291 showed differences in response to N forms and N levels, suggesting that different

292 species adopted distinctive strategies in response to N addition (Flores-Moreno *et al.*

293 2016; Broadbent *et al.* 2020).

294   The species responses to different N forms are related with the leaf N

295   concentration and RSR, which reflect the plant strategies of allocations to

296   aboveground and belowground tissues (Fortunel *et al.* 2012; Firn *et al.* 2019). When

297   exogenous N is added, it is expected that AGB increases with leaf N concentration

298   and decreases with RSR (Cobb *et al.* 2008; Lu *et al.* 2011; Kiær *et al.* 2013; Li *et al.*

299   2015). However, our study showed that as N addition increases, the correlations of

300   AGB with leaf N concentration and RSR only existed under NO_3^--N treatments but

301   not under NH_4^+-N treatments. The results indicate that the coupling of plant functional

302   traits and biomass was weakened by NH_4^+-N application, which may be due to the

303   disturbance of plant physiology by NH_4^+-N. Consistent with previous studies, NH_4^+-N

304   is a paradoxical nutrient ion, although it is a major N source for plants, it can result in

305   toxicity symptoms in many plants when cultured on NH_4^+-N as the exclusive N

306   source (van den Berg *et al.* 2008; Sarasketa *et al.* 2014; Hachiya *et al.* 2021). The

307   threshold of NH_4^+ toxicity depends on plant species (de Graaf et al. 1998; Britto &

308   Kronzucker 2002; Esteban et al. 2016). Above the threshold, NH_4^+ becomes toxic due

309   to multiple mechanisms. For instance, NH_4^+ inhibits plant uptake of essential cations

310   such as Ca^{2+}, Mg^{2+} and K^+ (Gloser & Gloser 2000). Immediate transformation of

311   NH_4^+ to N-rich amides and amino acids in plant cells suppresses the effective usage of

312   N by plant catabolism (Schortemeyer et al. 1997). In addition, the extensive H^+

313   release from the assimilation of NH_4^+ causes acidic stress on plant tissues (Hachiya et

314   al. 2021), in which situation plants invest energy to transport H^+ out of cells to

315   maintain cytoplasmic pH, further inhibiting plant growth (Gerendas & Ratcliffe

316   2000). When plants are poisoned by NH_4^+, their aboveground grows slowly, root

317 architecture is deformed, and root-shoot-ratio is decreased (Dominguez-Valdivia *et al.*

318 2008; Britto & Kronzucker 2013; Bittsanszky *et al.* 2015). In our study, *Astragalus*

319 *laxmannii* and *Potentilla chinensis* significantly decreased and even lost under NH_4^+-

320 N addition treatments, which was supported by studies that legumes and forbs show a

321 high sensitivity to NH_4^+ toxicity (Chaillou *et al.* 1986; van den Berg *et al.* 2008; Britto

322 & Kronzucker 2013).

323

324 **CONCLUSIONS**

325   N deposition has an important impact on grassland plant diversity. Our study

326 indicates that reduced and oxidated N affect grassland plant diversity differently.

327 Compared with dicotyledonous plants, monocotyledonous plants such as grasses and

328 sedges were more tolerant to NH_4^+ toxicity and had a competitive edge for light. Due

329 to the rapid transformation of reduced N to oxidated forms in the soil, reduced N

330 deposition decreases grassland plant biodiversity by both the physiological

331 intolerance to NH_4^+-N and the ecological competition among species, whereas the

332 impact of oxidated N is primarily related to the ecological competition (Fig. 6). As a

333 result, reduced N exerts greater impacts on grassland plant species losses than

334 oxidated N. Our results suggest that regions with greater NH_x/NO_y ratios of N

335 deposition may experience more severe biodiversity loss as N deposition continues to

336 increase. This study emphasizes that NH_x/NO_y ratio, in addition to the total N

337 deposition amount, should be considered as an important predictor of terrestrial

338 biodiversity loss.

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Table 1 Results of linear mixed models (LMMs) testing the effects of N forms (Fm) and levels (L) on the total numbers, total aboveground biomass (AGB) and species diversity in the mixed cultural system. F-test values (*F*) and P-values (*P*) are provided.

352

	Total numbers		Total AGB		Shannon-Wiener diversity		Simpson diversity	
	F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Fm	392	<.001	96.294	<.001	34.037	<.001	26.05	<.001
L	79.185	<.001	7.709	0.003	8.288	0.002	5.45	0.011
Fm × L	15.259	<.001	5.574	0.010	4.609	0.019	3.72	0.037

353

354 **Table 2 Results of LMMs testing the effects of N forms (Fm) and N levels (L) on the different species' PFTs in the monocultural system.**

355 AGB: aboveground biomass; BGB: belowground biomass; Height: plant height; LA: leaf area; SLA: specific leaf area; C/N: leaf C/N ratio;

356 RSR: root-shoot-ratio; SRL: specific root length; RTD: root tissue density; FRP: fine root proportion; Dm: root diameter. $*P < 0.05$; $**P < 0.01$;

357 $***P < 0.001$; ns: not significant.

358

Species	Variance	F-test											
		AGB	BGB	Height	LA	SLA	C/N	Leaf N	RSR	SRL	RTD	FRP	Dm
Leymus chinensis	Fm	12.86^{**}	25.64^{***}	64.73^{***}	88.41^{***}	1.39^{ns}	1.19^{ns}	1.44^{ns}	2.33^{ns}	0.00^{ns}	6.31^{*}	0.36^{ns}	7.00^{*}
	L	12.05^{***}	24.82^{***}	6.05^{**}	14.52^{***}	9.61^{**}	5.95^{**}	2.32^{*}	36.26^{***}	1.63^{ns}	0.65^{ns}	2.38^{ns}	3.16^{ns}
	Fm × L	9.31^{**}	29.85^{***}	4.55^{*}	8.15^{**}	2.20^{ns}	1.75^{ns}	1.13^{ns}	12.95^{***}	2.73^{ns}	1.11^{ns}	5.36^{*}	5.87^{**}
Astragalus laxmannii	Fm	62.37^{***}	14.87^{**}	172.98^{***}	138.53^{***}	127.65^{***}	2.34^{ns}	1.64^{ns}	0.00^{ns}	2.52^{ns}	0.19^{ns}	3.29^{ns}	0.17^{ns}
	L	2.83^{ns}	2.66^{ns}	8.81^{**}	1.96^{ns}	0.41^{ns}	4.42^{*}	10.29^{***}	3.74^{**}	11.91^{***}	3.43^{*}	1.36^{ns}	1.10^{ns}
	Fm × L	3.39^{*}	2.01^{ns}	10.1^{***}	5.2^{*}	3.00^{ns}	0.28^{ns}	0.59^{ns}	2.50^{ns}	5.28^{**}	2.17^{ns}	2.11^{ns}	2.18^{ns}
Carex korshinskyi	Fm	19.46^{***}	31.11^{***}	9.80^{**}	38.21^{***}	6.65^{*}	0.45^{ns}	0.00^{ns}	19.60^{***}	12.74^{**}	1.26^{ns}	73.57^{***}	0.13^{ns}
	L	4.24^{*}	7.50^{**}	3.26^{ns}	6.13^{**}	0.38^{ns}	10.67^{**}	8.28^{**}	31.92^{***}	4.44^{*}	1.05^{ns}	14.05^{***}	7.84^{**}
	Fm × L	2.07^{ns}	0.83^{ns}	1.68^{ns}	3.37^{*}	0.94^{ns}	0.24^{ns}	0.14^{ns}	2.84^{ns}	2.56^{ns}	1.84^{ns}	16.43^{***}	7.63^{**}
Potentilla chinensis	Fm	199.20^{***}	172.40^{***}	107.06^{***}	53.02^{***}	1.04^{ns}	15.84^{**}	16.31^{**}	44.78^{***}	0.21^{ns}	0.00^{ns}	2.3^{ns}	86.07^{***}
	L	2.39^{ns}	5.23^{*}	0.79^{ns}	3.09^{ns}	0.57^{ns}	8.34^{**}	11.42^{***}	14.08^{***}	3.49^{*}	0.78^{ns}	2.96^{ns}	1.57^{ns}
	Fm × L	8.63^{**}	1.73^{ns}	9.49^{**}	5.72^{**}	1.04^{ns}	0.48^{ns}	0.27^{ns}	0.48^{ns}	0.82^{ns}	1.16^{ns}	2.09^{ns}	3.39^{*}

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Figure legends

Figure 1 Responses of grassland species richness, Shannon-Wiener diversity and

evenness to N addition from the meta-analysis. (a) The overall responses and 95%

confidence intervals (CI) of species richness, Shannon diversity and evenness to

nitrogen (N) addition. (b) Effects of N addition on richness of grasses and non-grass

species. c-e: Dependences of species richness (c), Shannon-Wiener diversity (d) and

evenness (e) on N addition levels. The numbers of collected publications and the

sample sizes are shown in parentheses.

Figure 2 Effects of N forms (Fm) and levels (L) on the total plant number (a – b)

and total aboveground biomass (c – d) under the mixed cultural system. The

embedded plots in the panel (c) and (d) show the biomass without *Leymus chinensis*.

Bars represent mean ± S.E. (n = 3). The letters near the bars represent significant

differences among different N addition levels at $P < 0.05$.

Figure 3 Effects of N forms (Fm) and levels (L) on aboveground biomass of four

species in the monocultural system. (a) *Leymus chinensis*; (b) *Astragalus*

laxmannii; (c) *Carex korshinskyi*; and (d) *Potentilla chinensis*. Bars represent mean ±

S.E. (n = 3). Upper-case letters indicate differences among N levels, Lower-case

letters indicate differences among N forms and levels if interaction effect is

significant. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns: not significant.

Figure 4 Dependences of aboveground biomass on leaf nitrogen (N; a – b) and

385   **the root-to-shoot ratio (RSR; c – d).**

386

387   **Figure 5 Principal Component Analysis (PCA) based on plant functional traits**

388   **(PFTs) under NH$_4^+$-N (a) and NO$_3^-$-N (b) applications.** The percentage number

389   represents proportion of variation for which the axis accounts. AGB: aboveground

390   biomass; BGB: belowground biomass; C/N: leaf C/N ratio; Dm: root diameter; FRP:

391   fine root proportion; Height: plant height; RSR: root-shoot-ratio; RTD: root tissue

392   density; SLA: specific leaf area; SRL: specific root length.

393

394   **Figure 6 The summarized mechanisms of oxidized and reduced N in affecting**

395   **grassland plant biodiversity.** Oxidized N affects grassland plant biodiversity

396   primarily through inter-species interactions (i.e., mechanism 1 and 6), while reduced

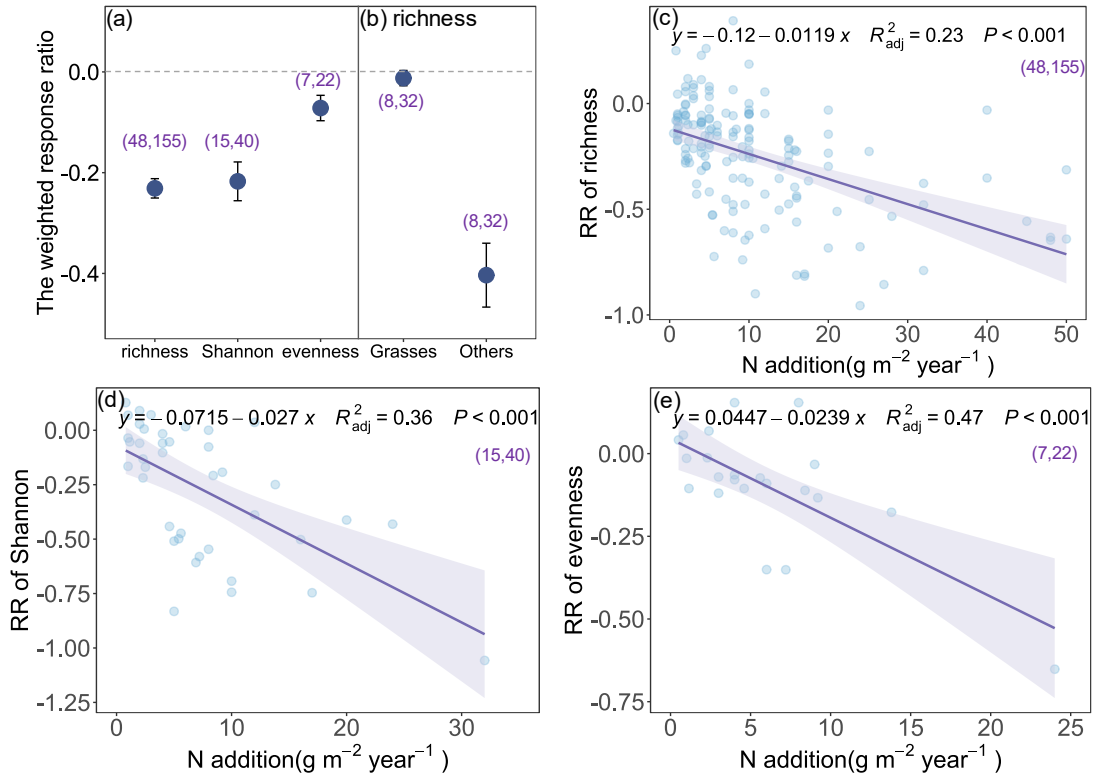397   N's effects include all six mechanisms.

Figure 1

404   **Figure 2**

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411 **Figure 3**

412

(a) *Leymus chinensis*

F_m** L*** $F_m \times L$**

(b) *Astragalus laxmannii*

F_m*** L^{ns} $F_m \times L$*

- Ammonium
- Nitrate

(c) *Carex korshinskyi*

F_m*** L* $F_m \times L^{ns}$

(d) *Potentilla chinensis*

F_m*** L^{ns} $F_m \times L$**

Aboveground biomass (g pot^{-1})

N addition rate (mg pot^{-1})

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420   **Figure 4**

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427   **Figure 5**

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(a) Ammonium

Leymus chinensis
Astragalus laxmannii
Carex korshinskyi
Potentilla chinensis

(b) Nitrate

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430
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Figure 6

439

Oxidized N deposition Reduced N deposition

NO$_y$ NO$_y$ NO$_y$ NH$_x$ NH$_x$ NH$_x$

① Light competition

② NH$_4^+$ toxicity

---Cation uptake decreased
---The effective usage of N
    suppressed
---Acidic stress increased
---Energy needed to matain pH
    increased

③ H$^+$ toxicity

④ Heavy metal toxicity

⑤ Base cations loss

species loss

Plant

Soil

⑥Seed richness decreased

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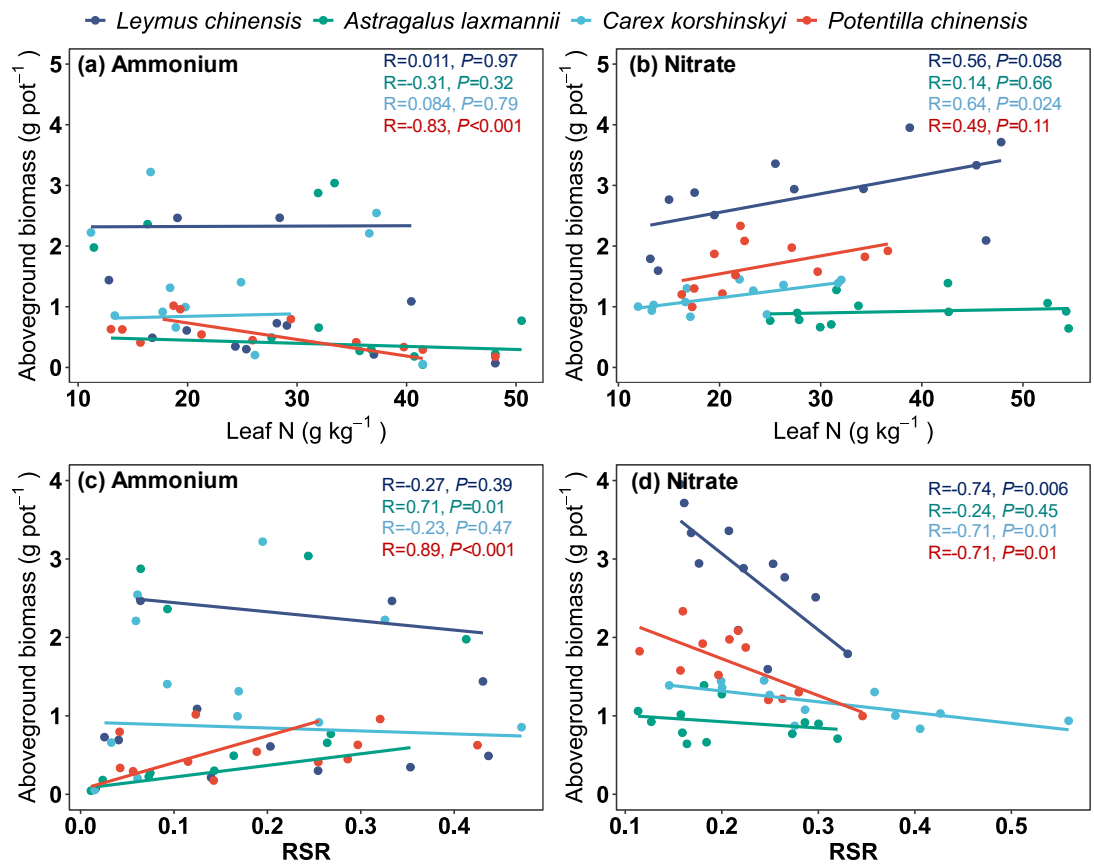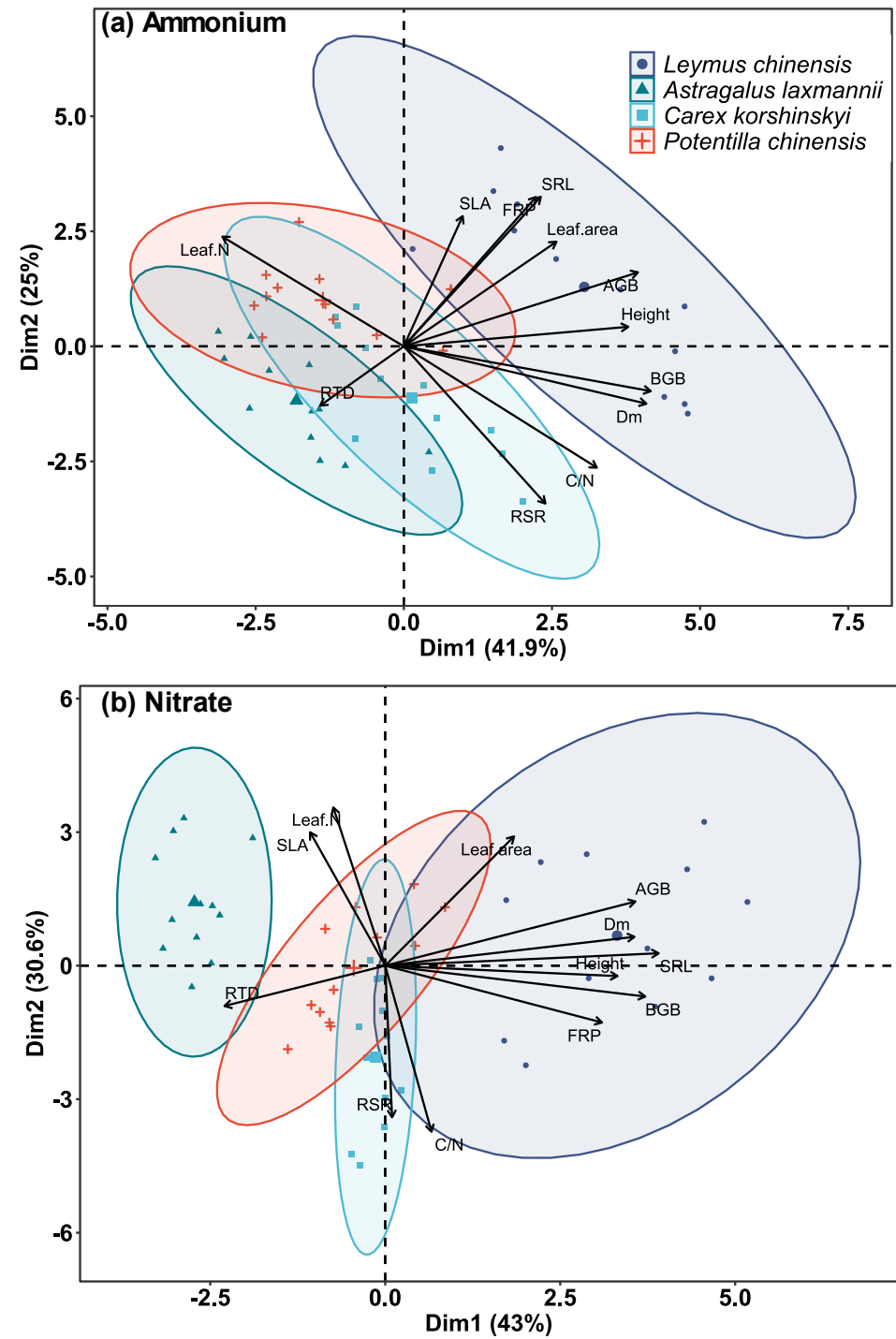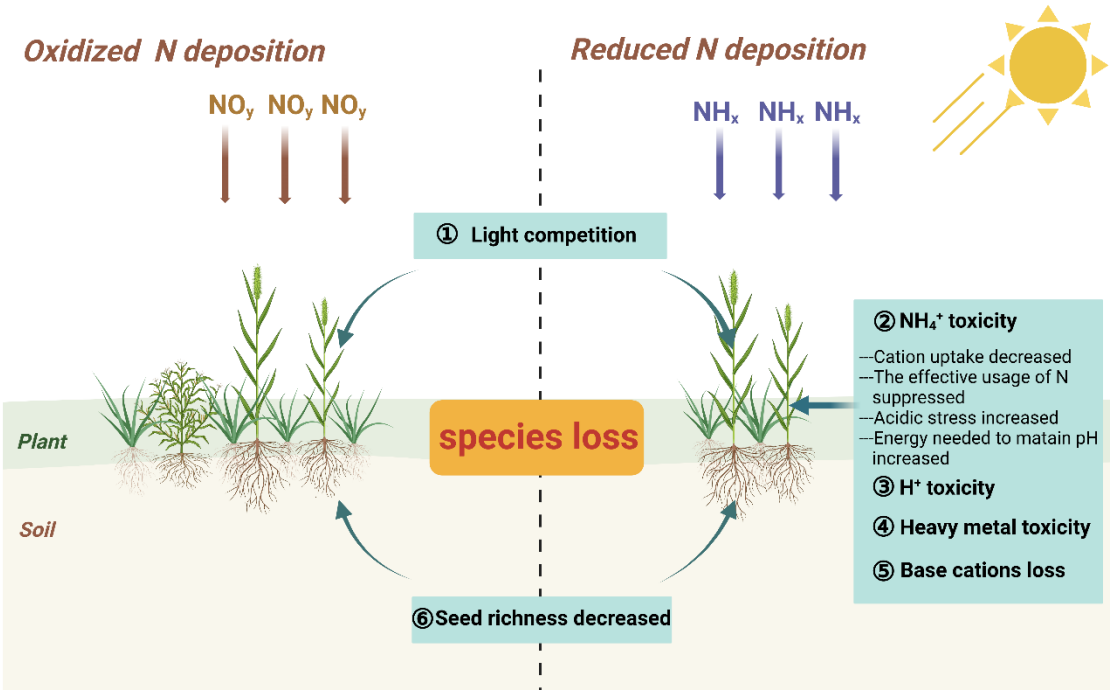
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