1 **Plant functional traits modulate the effects of soil acidification on above- and**

2 **belowground biomass**

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Abstract

Atmospheric sulfur (S) deposition has been increasingly recognized as a major driver of soil acidification. However, little is known on how soil acidification influences above- and belowground biomass via altering leaf and root traits. We conducted a 3-year S addition experiment to simulate soil acidification in a meadow. Grass (*Leymus chinensis* (Trin.) Tzvelev) and sedge (*Carex duriuscula* C. A. Mey) species were chosen to evaluate the linkage between plant traits and biomass. Sulfur addition led to soil acidification and nutrient imbalance. Soil acidification decreased specific leaf area (SLA) but increased leaf dry matter content (LDMC) in *L. chinensis,* showing a conservative strategy and thus suppression of aboveground instead of belowground biomass. Whereas in *C duriuscula,* soil acidification increased plant height and root nutrients (N, P, S, and Mn), favoring competition for natural resources by enhanced above- and belowground biomass, *i.e.*, adoption of an acquisitive strategy. Increased soil acidity resulted in an overall reduction in community aboveground biomass by 3-33%, but increased the community root biomass by 11-22% due to upregulation through higher soil nutrient availability. Our results demonstrate that plant above- and belowground biomass is affected by S-induced acidification. Understanding the linkage between plant biomass and functional traits contributes to better knowledge on plant-soil feedback in grassland ecosystems.

Keywords: sulfur addition, soil acidification, meadow grassland, functional traits, plant biomass

1 Introduction

Acid deposition as a consequence of anthropogenic activities has significant impacts on terrestrial biodiversity and ecosystem functions (Tian and Niu, 2015; Clark et al., 2019; Yang et al., 2021). Atmospheric sulfur (S) deposition is one of the main causes of soil acidification, with its effects equal to or exceeds that of nitrogen (N) deposition in Asia (Duan et al., 2016; Zhang et al., 2022). Despite a large decrease in average S deposition across China over the past decades, the problem is still very severe in Northeast China and Inner Mongolia (Yu et al., 2017). The northern grasslands of China as an integral part of the Eurasian grassland have experienced severe soil acidification with a significant decline in mean soil pH from 7.84 to 7.21 during 1980s- 2000s, and S deposition undoubtedly accelerates this process (Yang et al., 2012). Therefore, soil acidification has become a major global concern; it not only leads to soil nutrient imbalance but also decreases the productivity of terrestrial ecosystems (Chen et al., 2013a; Tibbett et al., 2019; Duddigan et al., 2021).

In natural ecosystems, sulfur is an essential nutrient element in forming plant proteins for being a constituent of certain amino acids (Vitousek and Howarth, 1991; Garrison et al., 2000). Shifts in plant species and community associated with S deposition are mainly a consequence of soil acidification rather than a S-fertilization effect (Clark et al., 2019). This is because soil pH is a primary regulator of nutrient availability that plant growth and species co-existence rely on (Bolan et al., 2003; Tibbett et al., 2019). For instance, soil acidification inhibits nitrification (Kemmitt et al., 2005), but facilitates the release of soil available phosphorus (P), micronutrients and the leaching of soil base cations (Jaggi et al., 2005; Chen et al., 2015; Feng et al., 2019). Evidence of a previous manipulative S addition experiment has shown that aboveground biomass (AGB) generally decreases with soil acidification, except high acid tolerance species sedges which displays a reversed pattern of responses in a subalpine grassland (Leifeld et al., 2013). The acidification-mediated decreases in soil cation concentrations (such as Ca^{2+} and NO_3^-) could increase the relative abundance of acid-tolerant and oligotrophic species (van Dobben and de Vries, 2010; Clark et al.,

62 2019), as a result of decreasing abundance of other species (Jung et al., 2018).

63 Additionally, soil Mn toxicity caused by soil acidification in calcareous grassland

64 asymmetrically reduces aboveground biomass of different species and functional

65 groups through suppression of photosynthesis (Tian et al., 2016).

66   Findings from a global meta-analysis predominantly on forest ecosystems show

67 negative acidification effects on root biomass under sulfuric acid addition (Meng et al.,

68 2019), because forest soils with low initial pH (pH < 5) generally have greater level of

69 Al^{3+} and Fe^{3+} but less base cations, hence the inhibition of root growth (Li et al., 2018).

70 Different from findings with forests, in typical and alpine grasslands belowground

71 biomass increases with soil acidification mainly due to the compensatory growth

72 concomitant with the dominance of graminoids over forbs (Chen et al., 2015; Wang et

73 al., 2020). Plausibly, perennial rhizome grasses and sedges have higher ionic tolerance

74 (such as H^+, Al^{3+}, NH_4^+, and SO_4^{2-}) than perennial bunchgrasses and forbs, which allow

75 for the maintenance of high community biomass under soil acidification (Chen et al.,

76 2015; Cliquet and Lemauviel-Lavenant, 2019; Wang et al., 2020). Therefore, shifts in

77 grassland community are mainly regulated by soil nutrient fluctuations as induced by

78 soil acidification that eventually affect above- and belowground biomass (Mitchell et

79 al., 2018; Wang et al., 2020).

80   Functional traits are known to substantially influence plant survival, growth and

81 reproduction via closely association with plant capability for resource acquisition

82 (Violle et al., 2007). To cope with environmental stresses for persistence and

83 reproduction, plants often rely on a possessing a broad spectrum of functional traits

84 ranging from conservative to proactive strategies of resource acquisition (De Battisti et

85 al., 2020). For example, some plant species can upregulate tissue nutrients as a fast

86 resource acquisition strategy when soil environmental conditions become challenging

87 (Mueller et al., 2012). In contrast, some plant species under environmental stresses tend

88 to be more nutrient-conservative by having long-lasting leaves generally with a low

89 specific leaf area (SLA) but a high leaf dry matter content (LDMC) (Kandlikar et al.,

90 2022). Grass species may also increase root length to avoid acid and Al^{3+} stresses

91 (Göransson et al., 2011). In general, studies show that species with an acquisitive

92   strategy gain a greater biomass more rapidly, but those with a conservative strategy
93   have a slower pace of biomass growth to prolong their life span (Reich, 2014; Hao et
94   al., 2020).

95   Due to difficulties in measuring grassland root traits in situ, we still have very
96   limited knowledge on how root trait strategies are linked to the response of
97   belowground processes to soil acidification. It is known that some plants can cope with
98   nutrient deficiency in acidic soils via modifications of their root morphologies and
99   through their nutrient uptake capability and metabolic adjustment (Hammond et al.,
100 2004). Plants growing in resource-poor soils tend to have lower specific root length
101 (SRL) and lower root nutrient concentrations for the conservation of resources
102 (Delpiano et al., 2020). Findings from a pot experiment show that root length of
103 perennial grasses decreases with soil acidification, demonstrating the constraint of root
104 development under environmental stresses (Haling et al., 2010). However, in natural
105 ecosystems, it has been found that grasses develop densely branched root systems with
106 higher nutrient use efficiency and are more stress-tolerant to nutrient deficiency for
107 maintaining nutrient balance and growth (Tian et al., 2022). Moreover, aboveground
108 and belowground biomass might be strongly influenced by specific functional traits
109 (Clark et al., 2019; Wang et al., 2020), soil nutrient availability, and nutrient contents
110 in leaves and roots under soil acidification (Geng et al., 2014; Rabêlo et al., 2018; Tian
111 et al., 2021). To date, it still remains elusive on how the functional traits for both above-
112 and belowground components of different species respond to soil acidification.

113   To understand how soil acidification resulting from S deposition influences plant
114 traits, biomass and the linkage between the two in coping with S-induced stress, we
115 conducted a S addition experiment with eight loading rates (from 0 to 50 g S m^{-2} yr^{-1})
116 to simulate different levels of soil acidification in a meadow grassland. We assessed the
117 role of plant above- and belowground traits and soil abiotic variables in affecting the
118 biomass of two dominant and contrasting plant species (*Leymus chinensis* (Trin.)
119 Tzvelev and *Carex duriuscula* C. A. Mey) under soil acidification. Specifically, we aim
120 to examine how the relationships between biomass and plant functional traits are
121 modified by changes in soil conditions. As a perennial rhizome grass, *L. chinensis* is

widely distributed in arid and semi-arid areas of northern China. This species occurs in the top layers of the local grassland communities, as such likely having an advantage in resource acquisition, especially for light. Additionally, grasses generally exhibit flexibility in absorbing various forms of soil N, thereby expanding their ecological niche (Grassein et al., 2015). The perennial rhizomatous sedge *C. duriuscula* is a shorter subordinate species and an indicator plant for soil degradation, and possesses cluster roots and tends to consume more photosynthetic products for increased ability to acquire nutrients (Zhang et al., 2021). Both species exhibit distinct rhizosheaths that enable them to tightly adhere to the soil and show compensatory growth in response to environmental disturbance (Tian et al., 2022). Our study addresses the following questions: (i) how do soil properties (*i.e.* soil pH, Ca^{2+}, Al^{3+}, available N, available P), above- and belowground plant traits (*i.e.* morphological and nutrient traits) and biomass respond to different doses of S addition in a meadow grassland? (ii) What are the key plant functional traits that regulate the biomass responses of two species to soil acidification? We hypothesized that soil acidification caused by S addition would lead to a nutrient imbalance in the meadow grassland soil. Grass *L. chinensis* may respond to soil acidification by adjusting its aboveground light acquisition traits for biomass growth; whereas the sedge *C. duriuscula* may adopt a strategy of the increased tissue nutrient concentrations for enhancing its tolerance to soil acidification, consequently leading to compensatory root growth (Fig. S1).

2 Materials and methods

2.1 Experimental site and design

This study was conducted at the Erguna Forest-Steppe Ecotone Research Station (50° 10′ N, 119° 23′ E) of Chinese Academy of Sciences in Inner Mongolia, China. The area belongs to a transitional climate zone between mid-temperate to cold-temperate climate with mean annual temperature and precipitation of -2.45 °C and 363 mm, respectively (Feng et al., 2019). Soil at the experimental site is classified as a Haplic Chernozem according to the Food and Agricultural Organization of the United Nations classification (IUSS Working Group WRB, 2014). and composed of 37 ± 0.9% sand,

151   $40 \pm 1.0\%$ silt and $24 \pm 0.8\%$ clay (Li et al., 2019). Vegetation in this area is a meadow

152   steppe community predominantly comprised of *L. chinensis*, *Stipa baicalensis* Roshev,

153   *Cleistogenes squarrosa* (Trin.) Keng, *C. duriuscula*, *Pulsatilla turczaninovii* Kryl. et

154   Serg., and *Cymbaria dahurica* Linn.

155   The experimental field had been used for hay harvesting until 2013, and was fenced

156   to exclude livestock grazing since then. A field elemental S addition experiment was

157   established in 2017, to simulate soil acidification caused by atmospheric S deposition

158   in a homogeneous and flat field containing naturally assembled communities. The

159   vegetation in the experimental plots is composed of the dominant species (relative

160   abundance >40%) *L. chinensis*, subordinate species (relative abundance between 1%

161   and 30%), including *S. baicalensis, C. duriuscula*, *C. squarrosa*, *Achnatherum*

162   *sibiricum* (Linn.) keng, *C. dahurica*, *P. turczaninovii*, *Thermopsis lanceolala* R. Br and

163   *Achnatherum sibiricum* (Linn.) keng. The experimental setup adopted a randomized

164   block design with eight levels of S addition rate (0, 1, 2, 5, 10, 15, 20, and 50 g S m^{-2}

165   yr^{-1}) and five replicated 6 m $\times$ 6 m plots (Fig. S2). The low dose S applications in our

166   study was to mimic the current atmospheric SO_4^{2-} deposition level (2 - 4 g S m^{-2} yr^{-1})

167   in Northeast of China (Yu et al., 2017). Adjacent plots were separated by a 2-m wide

168   and mowed buffer strip. Purified sulfur fertilizer (elemental S > 99%), mixed with 200

169   g soil collected from the untreated areas nearby, was applied by hand spreading

170   annually in late May starting in 2017. Sulfur powder in soil can be oxidized by soil

171   microorganisms to form H^+ and SO_4^{2-} which simulate soil acidification well (Duddigan

172   et al., 2021). In the present study, we collected plant and soil samples from all the plots

173   from five out of eight levels of S addition treatments (0, 5, 10, 20, and 50 g S m^{-2}yr^{-1}).

2.2 Plant and soil sampling

175   In early August 2019, the annual aboveground biomass of plant communities was

176   harvested at the peak growth. All living tissues were clipped within a randomly selected

177   1 m $\times$ 1 m quadrat in each plot, sorted by species and oven-dried at 65 °C for 48 h to

178   measurements of aboveground net primary productivity (ANPP) and leaf nutrient

179   concentrations.

We measured three aboveground morphological traits for the two dominant species *L. chinensis* and *C. duriuscula*. Ten individual plants with complete shoot were randomly selected for each species within each plot. They were measured for maximum height and then clipped at the ground level. All the samples were immediately placed in a portable refrigerator and later detached to measure leaf area in laboratory. To ensure water saturation of the leaves, the samples were submerged in purified water and rehydrated for a minimum period of 6 hours. Then the water-saturated leaves were carefully wiped off the surface water with filter paper and weighed. The leaf samples were all scanned using an electronic scanner (Eption Perfection V39, Seiko Epson Corporation, Japan) for measurements of leaf area and then dried to constant weight at 60 °C for 72 h for measurements of dry mass. Specific leaf area (SLA, cm^2 g^{-1}) was calculated as the ratio of projected leaf area to dry mass. Leaf dry matter content was calculated as the ratio of dry mass to saturated mass (LDMC, g g^{-1}).

Plant roots were sampled using the soil block method in late August 2019. Specifically, a 30 × 30 × 30 cm soil block was collected using a steel plate and a shovel from each plot, resulting in a total of 25 soil blocks. All soil blocks were immediately transported to the processing area upon collection and then gently loosened by hands to separate roots from soils. The separated plant roots were carefully washed to remove the adhering soil and placed in iceboxes for transportation to laboratory. Before determining root morphological and chemical traits, all root samples were frozen at -20 °C. At least 10 intact individual plants for each of *L. chinensis* and *C. duriuscula* within each plot were used for determination of root nutrient traits (root [N], [P], [S], [Ca], [Fe], and [Mn]) and morphological traits. The total root length, surface area and volume were determined using the scanned images by the WinRHIZO software (Regent Instruments Inc., Quebec City, QC, Canada). Specific root length (SRL, m g^{-1}) was calculated as total root length divided by its dry mass. Specific root surface area (SRA, cm^2 g^{-1}) was defined as total surface area divided by its dry mass. Root tissue density (RTD, g cm^{-3}) was obtained as the ratio of root dry mass to its volume. All the above samples were dried at 65 °C to constant mass for measurements of root biomass at species and community levels, respectively. Root and leaf N concentrations were

210   determined using an elemental analyzer (Vario EL III, Elementar, Hanau, Germany).

211   Both root and leaf samples were digested with 8 mL HNO_3 + 4 mL $HClO_4$ for

212   measurements of P, S, Ca, Fe and Mn concentrations by inductively coupled plasma

213   optical emission spectrometry (5100 ICP-OES; Perkin Elmer, America).

214   Fresh soil sampling (0 - 10 cm depth) was performed using a soil auger (5 cm inner

215   diameter). In each plot, three cores were collected and mixed into one composite sample.

216   After removing the visible plant detritus and rocks, we sieved the fresh soils through a

217   2-mm screen and divided each soil sample into two portions. For each sample, 10 g of

218   fresh soil was immediately extracted with 2 mol L^{-1} KCl solution. The extracted

219   solution was analyzed for nitrate (NO_3^-) and ammonium (NH_4^+) concentrations using

220   an autoAnalyser III continuous Flow Analyzer (Bran and Luebbe, Norderstedt,

221   Germany). The other subsamples were air-dried for measurements of physicochemical

222   properties. Soil pH was determined in 2.5: 1 (v/w) water/soil ratio with a digital pH

223   meter (Precision and Scientific Instrument Co. Ltd., Shanghai, China). Soil available P

224   concentration was measured using extractions in 0.5 mol L^{-1} $NaHCO_3$ solution and soil

225   available S concentration in 0.1 mol L^{-1} $Ca(H_2PO_4)_2$ solution (Tabatabai and Bremner,

226   1972) with a UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan) at 880 nm and

227   440 nm, respectively. Soil exchangeable aluminum (Al^{3+}) concentration was measured

228   using titration by 0.25 M NaOH to pH 7.0 after extraction with 1 M KCl solution from

229   air-dried soil samples. Soil exchangeable calcium (Ca^{2+}) was extracted by 1 M NH_4OAc

230   (pH = 7.0) at a 1:10 ratio (w/v) for 30 min. Diethylene triamine pentaacetic acid

231   (DTPA)-Fe and Mn were extracted from 10 g of air-dried soil sample with 20 ml of

232   0.005 M diethylenetriamine pentaacetic acid (DTPA), 0.01 M $CaCl_2$, and 0.1 M

233   triethanolamine (TEA) at pH 7.3 and determined using an atomic absorption

234   spectrophotometer (AAS, Shimadzu, Japan) (Feng et al., 2019; Li et al., 2021).

235   2.3 Statistical analyses

236   The effects of S addition on soil properties, plant traits and biomass were analyzed using

237   one-way analysis of variance (ANOVA) with Duncan test for comparison of multiple

238   means. Pearson's correlation analysis was performed to determine the relationships

239 among plant traits, plant biomass and soil abiotic variables across the S addition levels.

240 All the statistical analyses were performed using SPSS16.0 (SPSS Inc., Chicago, USA)

241 with significance level at $p < 0.05$.

242 We used structural equation modelling (SEM) to determine the indirect effects of

243 S addition in meditating the aboveground and root biomass from the perspective of

244 plant traits and soil factors. Prior to SEM analysis, the number of variables were

245 reduced by conducting principal component analysis (PCA) on all the variables for soil

246 (pH, NH_4^+-N, NO_3^--N, available P, available S, exchangeable cations Ca^{2+} and Al^{3+},

247 DTPA-Fe and DTPA-Mn), aboveground morphological traits (Height, SLA, LDMC),

248 leaf nutrient traits (Ca, Fe, Mn), root morphological traits (SRL, SRA, RTD) and root

249 nutrient traits (N, P, S, Ca, Fe, Mn). We then used the first principal components (PC1)

250 for the subsequent SEM analysis to represent soil acidification (PC1 explained 94.8%

251 of the variation), soil nutrients (PC1 explained 62.3% of the variation), root nutrient

252 traits in *C. duriuscula* (PC1 explained 45.7% of the variation), aboveground

253 morphological traits in *L. chinensis* (PC1 explained 54.7% of the variation) (Table S1).

254 A conceptual model of the hypothetical relationships was constructed (Fig. S1),

255 assuming that S addition would directly impact soil physicochemical properties, and

256 indirectly influence aboveground and belowground biomass through altering soil pH,

257 soil nutrient availability and plant traits. The SEM analyses were performed using

258 AMOS 24.0 (Amos Development Co., Maine, USA) and the PCA analyses were

259 performed using the vegan package in R 4.2.2.

260

261 **3 Results**

262 **3.1 Effects of S addition on soil properties**

263 Sulfur addition significantly decreased soil pH from 6.95 to 5.19, but increased soil

264 exchangeable Al concentration only in the highest S-addition level of 50 g S m^{-2} yr^{-1}

265 (Table 1). Similarly, S addition increased soil ammonium concentration but decreased

266 nitrate concentration in the highest S addition treatment (Table 1). Soil available P,

267 available S, DTPA-Fe and DTPA-Mn concentration increased with increasing S

268 addition rate, while soil exchangeable Ca concentration decreased (Table 1).

3.2 Effects of S addition on above- and belowground biomass

270 In the third year of the treatment, S addition suppressed the aboveground biomass of
271 plant community (Fig. 1). Aboveground biomass of the two dominant species showed
272 contrasting responses to S addition; it increased in *C. duriuscula* but decreased in *L.*
273 *chinensis* (Fig. 1). Moreover, S addition significantly increased the belowground
274 biomass of plant community owing to the increase in *C. duriuscula*, but had no impact
275 on the belowground biomass in *L. chinensis* (Fig. 1).

3.3 Effects of S addition on above- and belowground traits in *L.* *chinensis* and *C. duriuscula*

278 Sulfur addition resulted in increased plant height growth in *C. duriuscula*, but had no
279 impact in *L. chinensis* (Fig. 2a). The values of SLA were decreased, and the values of
280 LDMC increased in *L. chinensis* by the S addition treatment, but were not affected in
281 *C. duriuscula* (Fig. 2b and c). For the belowground tissues, S addition treatment
282 increased SRL in both species, but decreased SRA only in *C. duriuscula* (Fig. 2d and
283 e). However, RTD did not respond to the S addition treatment in either of the two
284 species (Fig. 2f).

285 For the nutrient traits, S addition had no significant impact on any of leaf [N], [P],
286 and [Ca], but increased leaf [S] and [Mn] in both species, and decreased leaf [Fe] in *C.*
287 *duriuscula* and increased leaf [Fe] in *L. chinensis* (Fig. 3). Root [N], root [P], root [S]
288 increased in *C. duriuscula* under the S addition treatment, but were not affected in *L.*
289 *chinensis* (Fig. 3h, i and j). Sulfur addition decreased root [Ca] in *C. duriuscula*, but did
290 not affect the root [Ca] in *L. chinensis* (Fig. 3k). Root [Fe] showed similar patterns of
291 responses to S addition treatments that of leaf [Fe]; it decreased in *C. duriuscula* and
292 increased in *L. chinensis* (Fig. 3l). Root [Mn] in both species were enhanced by S
293 addition (Fig. 3m).

3.4 Correlations and pathways of S-induced soil acidification effects on plant traits and biomass

Correlation analysis revealed species-specific responses to S addition in the aboveground morphological traits and the root nutrient traits (Figs. S3 and S4). This was mainly due to an increase in soil acidity, and occurrence of Al^{3+} toxicity and nutrient imbalance (*i.e.*, the deficient or excessive level of certain nutrients in the soil) induced by S addition, as demonstrated by the well fitted structural equation modelling (SEM) (χ^2 =51.83, *P* = 0.10, df = 40, AIC = 103.83, n = 25) (Fig. 4). The indirect positive effect of S addition on community belowground biomass was mainly imposed through a decreased soil pH in combination with an imbalance of soil available nutrients, alteration of leaf and root nutrient traits, and increased belowground biomass in *C. duriuscula*, collectively accounting for 69% of the variation in community belowground biomass (Fig. 4). The indirect negative effect of S addition on community aboveground biomass was mainly operated through soil acidification, changes in the aboveground morphological traits and increased aboveground biomass in *L. chinensis*, altogether accounting for 59% of the variation in community aboveground biomass (Fig. 4).

4. Discussion

4.1 Species-specific trait responses to S addition

The patterns of trait response to S addition differed between *L. chinensis* and *C. duriuscula*. Specifically, the nutrient traits in *L. chinensis* were less plastic, as evidenced by unchanged concentrations of N, P, S, and Ca, compared with *C. duriuscula*. *Leymus chinensis* was suggested to be a highly homoeostatic species with greater stability in elemental composition in a temperate steppe (Yu et al., 2010). Higher macroelement homeostasis helps plant maintain the functional and growth stability to resist changes in soil environment (Yu et al., 2010; Feng et al., 2019).

It is intriguing to note that increases in both leaf and root [Fe] in *L. chinensis* caused by S addition were not associated with soil available [Fe] (Figs. 3 and S3). Iron uptake and assimilation has been shown to be dependent on sulfate availability (Zuchi et al., 2012). Previous research demonstrated close relationships between Fe and S nutrition, suggesting common regulatory mechanisms for the homeostasis of the two elements

(Forieri et al., 2013). For grasses, S addition could enhance assimilation of plant S, which is subsequently incorporated into methionine in order to accelerate the secretion of phytosiderophore (Zuchi et al., 2012; Courbet et al., 2019). However, Fe absorption in *C. duriuscula* was inhibited by soil acidification, consistent with Fe (III)-reduction-based mechanism (Tian et al., 2016). Namely, acquisition of Fe by non-graminaceous monocotyledonous species is mediated by the reduction of Fe^{3+} to Fe^{2+} catalyzed by the ferric chelate reductase in root cells; Fe^{2+} absorption can be further interfered by competition with Mn^{2+} for the same metal transporter (Curie and Briat, 2003; Pittman, 2005). Acidification-induced higher soil DTPA-Mn concentration in the calcareous soil contributed to Mn accumulation in plant tissues of the two species (Figs. 3 and 5). Sulfur addition increased tissue [Mn] greater in *C. duriuscula* than in *L. chinensis*.

Leymus chinensis responded to soil acidification stress by decreasing SLA and increasing LDMC to reduce the loss of water and nutrients, adopting a conservative resource-uptake strategy. The SLA and LDMC in *L. chinensis* were significantly correlated with soil exchangeable Al, implying that conservative traits might also be linked with Al-resistant strategy (Poozesh et al., 2007). We found that with decreasing soil pH, soil nitrate was reduced and was positively correlated with SLA but negatively with LDMC in *L. chinensis* (Table 1 and Fig. S3). Soil nitrification has been shown to be positively related to leaf traits (such as leaf [N] and SLA; Laughlin et al., 2011). This suggests that a decrease in soil nitrate under soil acidification could be an important driver of plant trait variations. For *L. chinensis*, the belowground traits were unsusceptible to S addition compared with *C. duriuscula*. One plausible explanation for this insensitivity is that deep-rooted plants are more resilient to changing soil environment than the shallow-rooted plants (such as sedge *C. duriuscula*) (Zhang et al., 2019). We found both species responded to S-induced soil acidification by enhancing SRL, in agreement with findings by Göransson et al. (2011) that grass species increases root length to avoid acid stress. These results indicate that variation of root morphological traits has the potential to mitigate the negative effects of soil acidity and should be considered as part of stress-avoidance or tolerance strategies (Thomaes et al., 2013).

4.2 Species-specific and community biomass responses to S addition

To clarify the underlying mechanisms of the regulation of plant biomass by functional traits, we explored the role of morphological and nutrient traits in mediating aboveground and belowground biomass changes under S addition. We found that the aboveground and root traits in *L. chinensis* and *C. duriuscula* exhibited contrasting adaptive strategies for acquiring aboveground and belowground resources (Figs. 4 and 5). SEM illustrated that a decrease in aboveground biomass in *L. chinensis* was related to increased soil acidification and the conservative responses in aboveground morphological traits under S addition (Figs. 4 and 5). *Leymus chinensis* is found to be a nitrophilic and resource-acquisitive species under N-rich environment (Feng et al., 2019; Yang et al., 2019), but is disadvantaged under S-induced soil acidification. For example, we found SLA and LDMC in *L. chinensis* were positively correlated with the aboveground biomass of both *L. chinensis* and plant community (Fig. S3). Soil acidification has been found to result in enhanced toxic effects of proton and exchangeable Al (Roem and Berndse, 2000). In view of environmental stress hypothesis, plant species could adopt different strategies in term of trait responses to mitigate such environmental stress (Encinas-Valero et al., 2022). SLA and LDMC are often believed to be prominent indicators of plant strategy for maintaining productivity in response to environmental stress and disturbance regimes. Stress tolerant species normally have lower growth rates, photosynthetic rates, and SLA but higher LDMC (Pérez-Harguindeguy et al., 2013). Sulfur addition causes acidic stress to plants, leading to reduced SLA accompanied with lower photosynthesis and decreased plant aboveground productivity. Damages to photosynthetic capacity associated with oxidative stress has been found to occur in tree species under acid rain (Chen et al., 2013b), yet it is still less understood how plants in grassland ecosystems respond physiologically and biochemically to soil acidification. The future research needs to test the photosynthetic and antioxidant responses of plants to soil acidification.

We found that plant community aboveground biomass exhibited a trend of decline from 22% to 11% under soil acidification, although the overall effect was rather weak

between pH 6.7 and 5.19 (Fig. 1, Table 1). In this study, *Leymus Chinensis* was found to play a dominant role contributing to aboveground productivity, consistent with the findings by Tibbett et al. (2019) that grasses make up about 60% of plant coverage in acid grassland and Heathland. Therefore, the decreased aboveground biomass in *L. chinensis* was one of the reasons for the decline of community aboveground productivity (Fig. 4). Another explanation for the decline of aboveground biomass may be competitive exclusion of bunchgrasses and forbs under soil acidification (Stevens et al., 2010; Chen et al., 2015). Together, findings from this study help advance our knowledge on the importance of leaf morphological traits in regulating grassland productivity in response to soil acidification.

In contrast to the role of *L. Chinensis*, *C. duriuscula* was found to dominate the influence on belowground biomass of plant community under soil acidification; the belowground biomass in both *C. duriuscula* and plant community significantly increased from 19 to 52% by S addition (Fig. 1). The sedge species (e.g. *C. duriuscula*) appears to be more tolerant to S-induced soil acidification than the perennial rhizome grass species (e.g. *L. chinensis*). This finding is partly supported by results for alpine grasslands and typical steppe ecosystems (Chen et al., 2015; Wang et al., 2020). Previous studies suggested that the sedge had a greater competitive advantage in nutrient-poor environments than other functional groups (Gusewell, 2004). An increase in root biomass under soil acidification suggested that sedge invested more resources in favor of nutrient acquisition. SEM provided further evidence that for *C. duriuscula*, the higher nutrient demand (such as root [N], [P], [S], [Mn]) was matched by greater root biomass under S addition treatment (Fig. 4). An increased root biomass in *C. duriuscula* contributed to increased belowground biomass of plant community as a result of shifts in soil available nutrients under S addition. Our study provides direct evidence that *C. duriuscula* is highly efficient in nutrient acquisition and thereby responds to soil acidification stress with increased biomass production (Figs. 4 and 5). Our short-term findings suggest that sedges play an important role in preventing the decline of grassland productivity in acidified soils. Consistent with results from a long-term acidification experiment (Tibbett et al., 2019), compensatory growth of acid-

tolerant species is probably key to maintaining grassland productivity over the longer term, particularly for ecosystems that experience acidification by chronic N and S deposition.

For grassland ecosystems, most of the carbon is allocated belowground (Bontti et al., 2009). Accumulation of roots may benefit competition for nutrient and water resources in a short term (Wang et al., 2020). In the longer term, however, asymmetric light competitive advantage of larger individuals (*L. chinensis*) rather than competition for soil resources (DeMalach and Kadmon, 2017) would likely have a decisive effect on grassland plant community productivity and diversity under soil acidification. Findings in this study are in contrary to previous findings by Wang et al. (2020), who reported that diameter of 3rd-order roots contributed to the increased community belowground biomass under soil acidification in an alpine grassland. Our study clearly demonstrates that leaf and root nutrients as a whole jointly mediate the community belowground biomass in response to soil acidification induced by S addition.

5 Conclusion

Our results highlighted that the aboveground and root traits played important roles in mediating grassland plant competition for environment resources under soil acidification. Sulfur addition resulted in soil acidification, and leads to nutrient imbalance (i.e. higher ammonium, available P, Fe, Mn and exchangeable Al^{3+}, but lower nitrate and exchangeable Ca^{2+}). The dominant species *L. chinensis* showed a conservative trait response strategy, with decreased SLA and increased LDMC under S addition. Moreover, the conservative traits were linked with stable root biomass but lower aboveground biomass as a direct impact from soil acidification. In contrast, *C. duriuscula* displayed an efficient nutrient acquisitive strategy, with increased plant height and more efficient root traits ([N], [P], [S], [Mn], SRL) in favor of aboveground and root productivity under S addition, as mediated via altered soil acidity and nutrient availability. Such divergent and species-specific responses were strongly driven by soil environmental conditions which resulted in inconsistent changes in grassland community aboveground and belowground biomass under S addition. Our results

highlight the important roles of both aboveground and root traits in regulating species and community biomass under soil acidification.

Author contributions. YJ conceived the study and designed the experiment. TL and HL conducted the experimental work and the data analysis. XF wrote the manuscript with substantial editing by RW, JC and YJ.

Competing interests. None of the authors have a conflict of interest.

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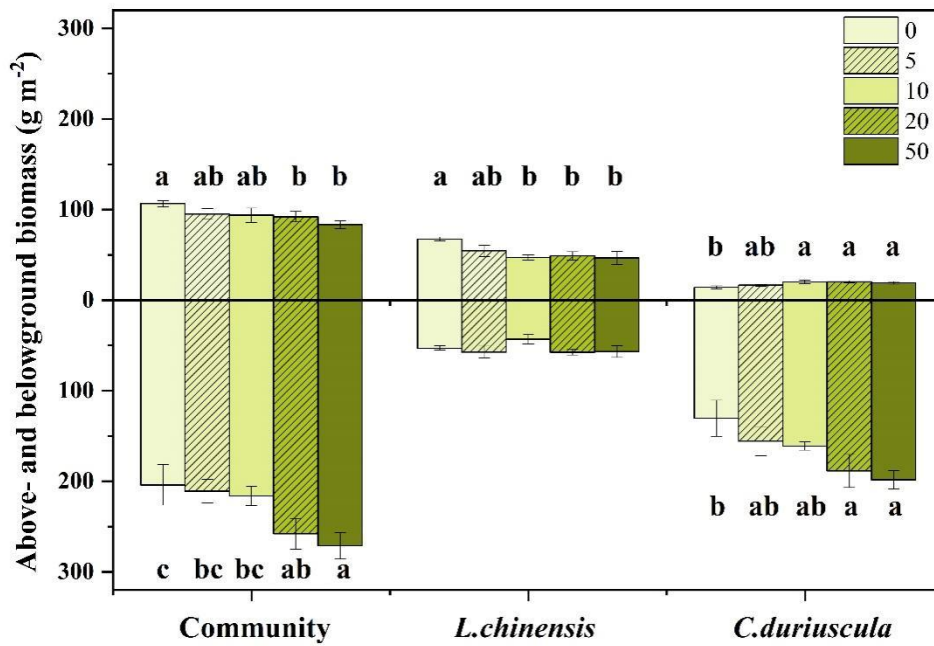
709 **Figures**

710

711 **Fig. 1** Effects of S addition on community and species aboveground and belowground

712 biomass. Bars are means ± the standard error. Lower case letters indicate significant

713 difference among treatments ($P < 0.05$).

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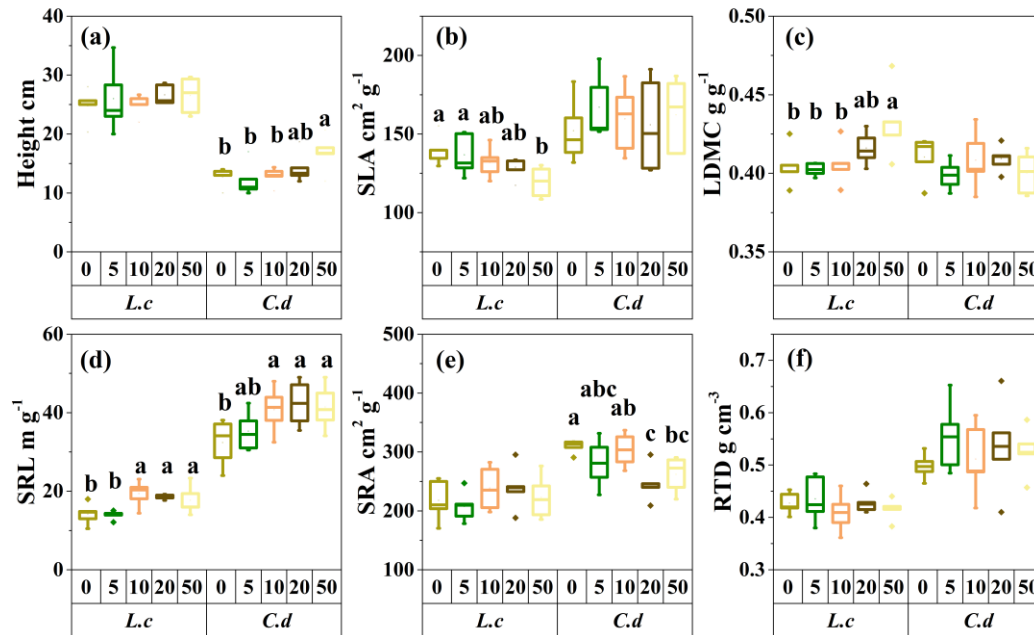
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717 **Fig. 2** The response of the morphological traits to S addition for the two dominant

718 species in a meadow steppe. Abbreviations: SLA, Specific leaf area; LDMC, Leaf dry

719 matter content; SRL, specific root length; SRA, specific root area; RTD, root tissue

720 density; *L.c*, *L. chinensis*; *C.d*, *C. duriuscula*. Different letters above the bars indicate

721 significant influence among the S-addition level by one-way ANOVA at *P* < 0.05.

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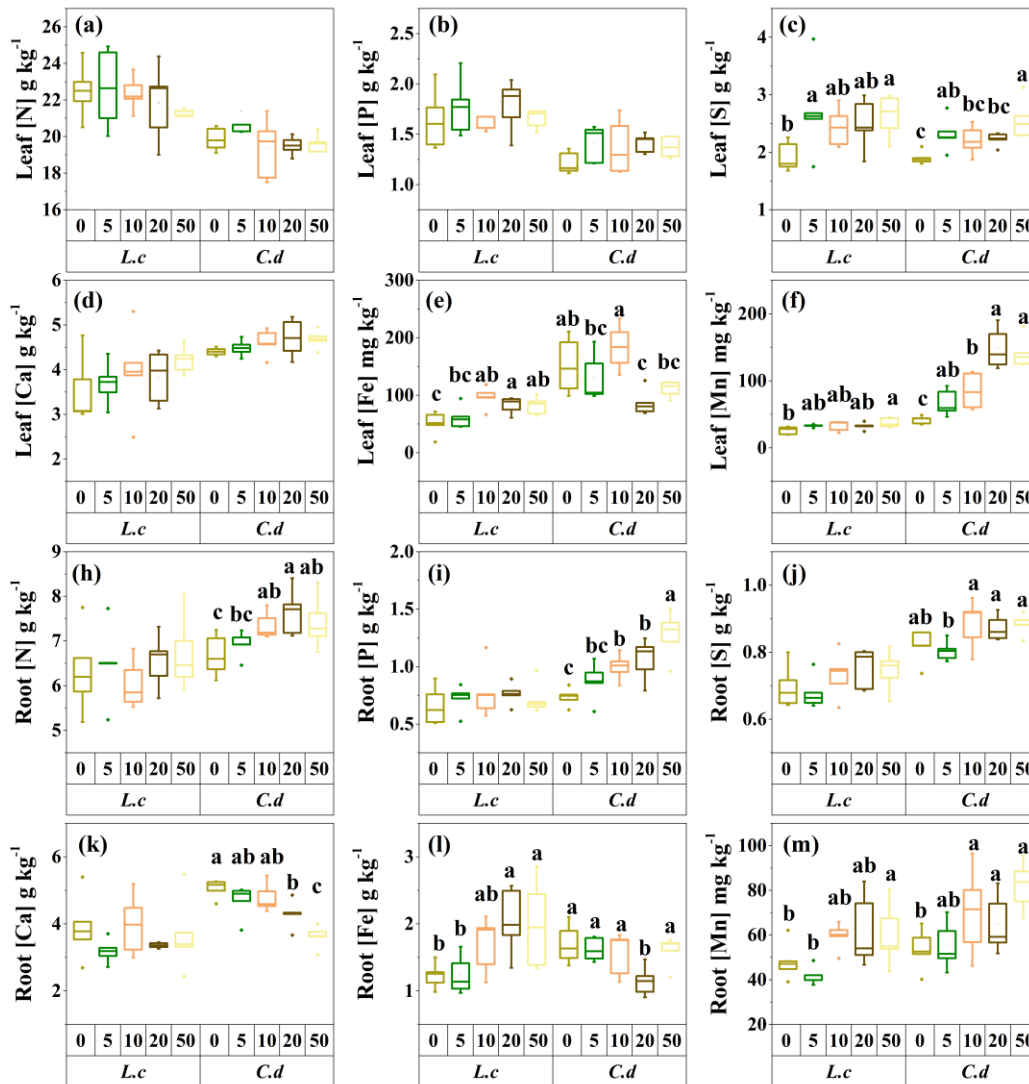
Fig. 3 The response of the chemical traits to S addition for the two dominant species in a meadow steppe. Abbreviations: Leaf [N], leaf N concentration; Leaf [P], leaf P concentration; Leaf [S], leaf S concentration; Leaf [Ca], leaf Ca concentration; Leaf [Fe], leaf Fe concentration; Leaf [Mn], leaf Mn concentration; Root [Ca], root Ca concentration; Root [Fe], root Fe concentration; Root [Mn], root Mn concentration; Root [N], root nitrogen concentration; Root [P], root phosphorus concentration; Root [S], root sulfur concentration; *L.c*, *L. chinensis*; *C.d*, *C. duriuscula*. Different letters above the bars indicate significant influence among the S-addition level by one-way ANOVA at $P < 0.05$.

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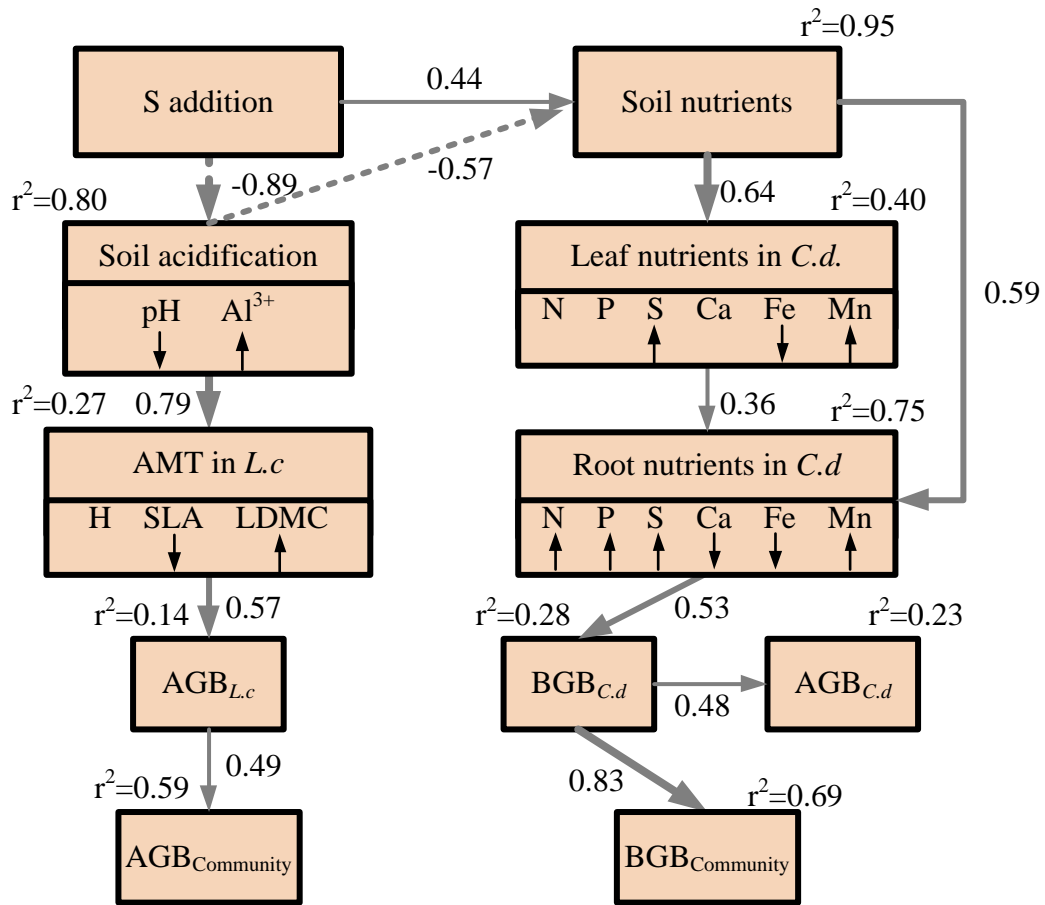
Fig. 4 Structural equation model of S addition on plant community biomass through the plausible pathways. Square boxes indicate the included variables in the analysis: S addition; Soil nutrients include soil NH_4^+-N and NO_3^--N concentrations, soil available phosphorus, soil available sulfur; soil exchangeable cations Ca^{2+}, Fe^{2+} and Mn^{2+}; soil acidification includes soil pH and exchangeable Al^{3+}; Aboveground morphological traits (AMT) includes plant height, specific leaf area, leaf dry matter content *in L. chinensis*; Leaf nutrients include leaf N, P, S, Ca, Fe, Mg concentrations in *C. duriuscula*; Root nutrients include root N, P, S, Ca, Fe, Mg concentrations in *C. duriuscula*; *C. duriuscula* aboveground biomass; *C. duriuscula* belowground biomass; *L. chinensis* aboveground biomass; Community aboveground biomass and belowground biomass. The symbols ↓ and ↑ indicate significant decrease or increase, respectively, with increasing S addition. The final SEM adequately fitted the data: $\chi^2 = 51.83$, DF = 40, $P = 0.10$, AIC = 103.83, n=25. R^2 values next to each response variable indicate the proportion of variation explained by relationships with other variables.

751 Solid and dashed arrows represent significant positive and negative pathways ($P < 0.05$),

752 respectively. Nonsignificant ($P > 0.05$) pathways are not shown. Values at each arrow

753 indicate the standard path coefficient, which is equivalent to the correlation coefficient.

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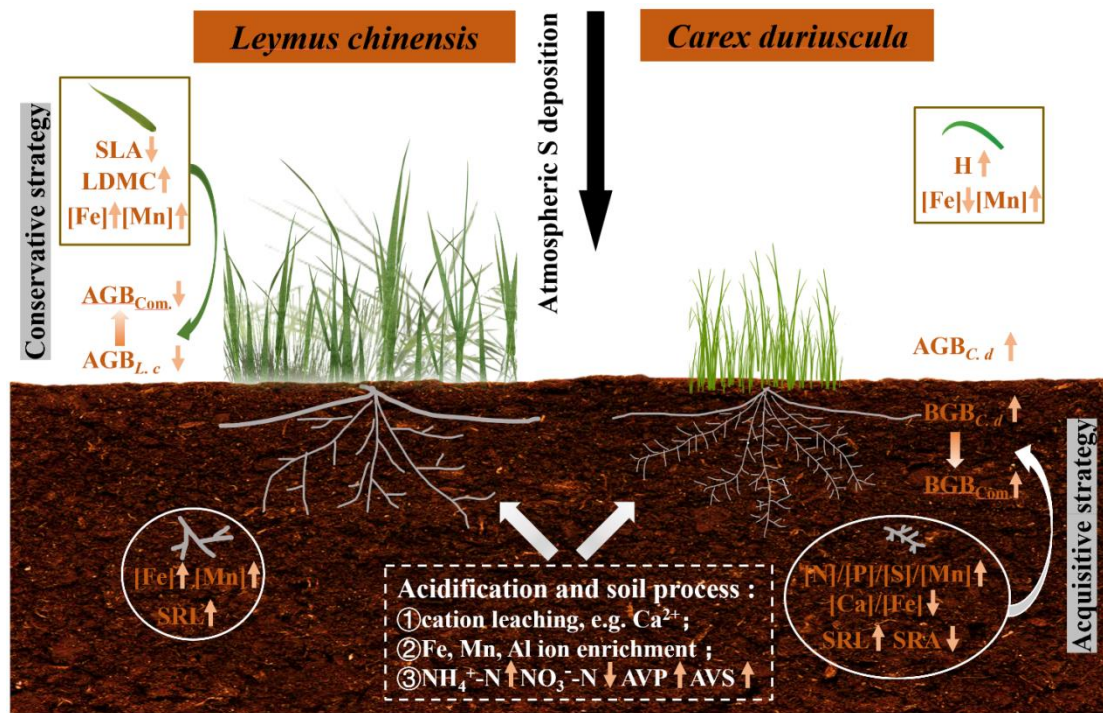
756

757 **Fig. 5** Schematic diagram illustrating the ecological effects of S-induced soil

758 acidification on above- and belowground biomass and traits of two dominant species in

759 a meadow steppe. ↑ = increase in response to S addition; ↓ = decrease in response to S

760 addition; Com. = Community; AVP = Soil available phosphorus; AVS = Soil available

761 sulfur.

762

763 **Table**

764 **Table 1** Effects of S addition on soil abiotic variables. All numbers refer to the mean

765 (the standard error). Lower case letters indicate significant difference among treatments

766 ($P < 0.05$).

Soil parameters	S addition				
	0	5	10	20	50
Soil pH	6.95(0.06) a	6.70(0.07) ab	6.77(0.17) a	6.17(0.31) b	5.19 (0.20) c
Ex. Al^{3+}	5.49(0.72) b	5.49(0.18) b	6.84(0.45) b	9.09(1.44) b	20.07(3.24) a
Ammonium	4.76(0.31) b	4.36(0.08) b	4.92(0.68) b	4.67(0.22) b	8.33(1.73) a
Nitrate	4.88(0.42) a	5.44(0.73) a	5.45(1.01) a	4.60(0.95) a	1.41(0.31) b
AVP	5.20(0.64) b	5.27(0.71) b	4.58(0.35) b	6.94(0.60) a	7.08(0.38) a
AVS	8.78(0.78) c	10.30(1.33) c	15.09(1.89) c	40.64(8.56) b	114.41(6.85) a
DTPA-Fe	22.10(1.14) c	27.94(0.02) bc	30.62(0.02) bc	38.07(0.04) b	58.72(0.07) a
DTPA-Mn	19.26(1.56) c	27.43(1.43) bc	33.23(3.10) bc	41.66(4.40) b	79.60(7.54) a
Ex. Ca^{2+}	22.12(0.54) a	20.66(0.90) ab	20.14(1.09) ab	19.17(0.90) b	18.50(0.61) b

767 Note: Ex. Al^{3+}: Exchangeable Al^{3+}, mg kg^{-1}; Ammonium: soil NH_4^+-N concentration, mg kg^{-1};

768 Nitrate: soil NO_3^--N concentration, mg kg^{-1}; AVP: soil available phosphorus, mg kg^{-1}; AVS: soil

769 available sulfur, mg kg^{-1}; DTPA-Fe: Soil DTPA-Fe concentration, mg kg^{-1}; DTPA-Mn: Soil DTPA-

770 Mn concentration, mg kg^{-1}; Ex. Ca: Exchangeable Ca^{2+}, cmol kg^{-1}.

771