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Plant functional traits modulate the effects of soil acidification on above- and belowground biomass

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Abstract

Atmospheric sulfur (S) deposition has been extensively recognized as a major driving force of soil acidification. However, little is known on how soil acidification influences above- and belowground biomass via altering leaf and root traits.

A 3-year elemental S addition were conducted to simulate soil acidification in a meadow. Grass (*Leymus chinensis*) and sedge (*Carex duriuscula*) species were chosen to demonstrate the linkage between plant traits and biomass.

Sulfur addition led to soil acidification and nutrient imbalance. For *L. chinensis*, soil acidification decreased specific leaf area but increased leaf dry matter content showing a conservative strategy and thus suppression of aboveground instead of belowground biomass. For *C duriuscula*, soil acidification increased plant height and root nutrients (N, P, S, and Mn) for competing resources by investing more on above- and belowground biomass, *i.e.*, an acquisitive strategy. An overall reduction in community aboveground biomass by 3-33% was resulted from the increased soil acidity. While the community root biomass increased by 11-22% as upregulated by higher soil nutrient availability.

Our results provide new insights that plant above- and belowground biomass is conditioned by S-invoked acidification and their linkages with plant traits contributed to a deeper understanding of plant-soil feedback.

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

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1 Introduction

Acid deposition as a consequence of anthropogenic activities will have important impacts on terrestrial biodiversity and ecosystem functions and services (Tian and Niu, 2015; Clark et al., 2019; Yang et al., 2021). Atmospheric sulfur (S) deposition is one of the main causations of soil acidification, and its contribution is equal to or exceeds that of nitrogen (N) deposition in Asia (Duan et al., 2016; Zhang et al., 2022). Despite large decrease in average S deposition across China over the past decades, it is still very serious 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 an overall decrease of 0.63 pH units, while S deposition can undoubtedly accelerate this process (Yang et al., 2012). Therefore, soil acidification has become a major global concern, not only leading to soil nutrient losses but also decreasing the productivity of terrestrial ecosystems (Chen et al., 2013; Tibbett et al., 2019; Duddigan et al., 2021).

In natural ecosystems, S limitation rarely occurred (Vitousek and Howarth, 1991; Garrison et al., 2000). Shifts in plant species and community associated with S deposition were 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., 2002), but promotes the release of soil available phosphorus (P), micronutrients and the leaching of soil base cations (Jaggi et al., 2001; Chen et al., 2015; Feng et al., 2019). Evidence from contrived S addition experimentation has shown that aboveground biomass (AGB) decreased with soil acidification, whereas sedges with high acid tolerance revealed the opposite pattern in a subalpine grassland (Leifeld et al., 2011). The acidification-mediated decrease 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., 2019) as a result of decreasing abundance of other species (Jung et al., 2018). Additionally, soil Mn toxicity caused by soil

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acidification in calcareous grassland asymmetrically curbed aboveground biomass of different species and functional groups through suppression of photosynthesis (Tian et al., 2016).

A global meta-analysis with most data from forest ecosystems found negative acidification effect on root biomass under sulfuric acid addition (Meng et al., 2019). This was because forest soils with low initial pH (pH < 5) generally experienced greater Al^{3+} and Fe^{3+} but less base cations, thus inhibiting root growth (Li et al., 2018). Different from findings in forests, belowground biomass increased with soil acidification in typical and alpine grasslands which was mainly due to the compensatory growth concomitant with graminoids dominating over forbs (Chen et al., 2015; Wang et al., 2020). Possibly, perennial rhizome grasses and sedges have higher ionic tolerance (such as H^+, Al^{3+}, NH_4^+, and SO_4^{2-}) than perennial bunchgrasses and forbs, which allowed for the maintenance of high community biomass under soil acidification (Chen et al., 2015; Cliquet and Lemauviel-Lavenant, 2019; Wang et al., 2020). Therefore, shifts in grassland community are mainly regulated by soil nutrient fluctuations as induced by soil acidification that eventually affect above- and belowground biomass (Mitchell et al., 2018; Wang et al., 2020).

Functional traits substantially influence plant survival, growth and reproduction via closely associating with plant capability of resource acquisition (Violle et al., 2007). Coping with environmental stresses to persist and reproduce, plants rely on a combination of different functional traits ranging from conservative to acquisitive strategies of resource acquisition (De Battisti et al., 2020). For example, some species upregulate tissue nutrients as a fast resource acquisitive strategy when soil environmental conditions become challenging (Mueller et al., 2012). On the opposite, some plant species under environmental stresses tend to be more nutrient-conservative by developing long-lasting leaves generally with a low specific leaf area (SLA) but a high leaf dry matter content (LDMC) (Kandlikar et al., 2004). Grass species may also increase root length to avoid acid and Al^{3+} stresses (Göransson et al., 2010). In general, species with acquisitive strategy accumulate greater biomass in a rapid way, but species

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with conservative strategy slow down biomass growth to elongate their life span (Reich, 2014; Hao et al., 2020).

Due to difficulties in measuring grassland root traits in situ, our understanding is very limited in terms of using root trait strategy to explain the response of belowground processes to soil acidification. A pot experiment found that root length of perennial grasses decreased with soil acidification, demonstrating the constraint of root development in stressful circumstances (Haling et al., 2010). Additionally, aboveground and belowground biomass might also strongly and complicatedly be influenced by specific functional traits (Clark et al., 2019; Wang et al., 2020), soil nutrient availability, and nutrient contents and interactions in leaves and roots under soil acidification (Geng et al., 2014; Rabêlo et al., 2018; Tian et al., 2021). Overall, it still remains elusive for how soil acidification can influence the bottom-up pathway of "soil variables-functional traits-plant biomass".

To understand how soil acidification influences plant traits, biomass and their relationships, we conducted a S addition experiment that included eight rates (from 0 to 50 g S m^{-2} yr^{-1}) to simulate soil acidification in a semiarid grassland. We assessed the role of plant above- and belowground traits and soil abiotic variables in driving the grassland biomass of two dominate species (*Leymus chinensis* and *Carex duriuscula*) under soil acidification. Specifically, we also aimed to quantify how these relationships were modified by changes in soil conditions and related trait response strategy. We addressed 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 rates of S addition in the meadow grassland? (ii) What are the key traits that correlate with the biomass responses of two species to soil acidification? We hypothesize that S addition would asymmetrically affect the aboveground vs. belowground biomass in a species-specific way due to disparate trait responses of two dominate species to soil acidification (Fig. S1).

2 Materials and methods

2.1 Experimental site and design

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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 in the experimental site is classified as a Haplic Chernozem according to the Food and Agricultural Organization of the United Nations classification and composed of 37 ± 0.9% sand, 40 ± 1.0% silt and 24 ± 0.8% clay. Vegetation in this area is a meadow steppe community including common plant species of *Leymus chinensis*, *Stipa baicalensis*, *Cleistogenes squarrosa*, *Carex duriuscula*, *Pulsatilla turczaninovii*, and *Cymbaria dahurica*.

A field elemental S addition experiment was established in 2017 to simulate soil acidification caused by atmospheric S deposition. A randomized block design was exploited included eight levels of S addition (0, 1, 2, 5, 10, 15, 20, and 50 g S m^{-2} yr^{-1}), and each treatment had five replicates. The low dose S applications in our study was to imitate the current atmospheric SO_4^{2-} deposition level (2 - 4 g S m^{-2} yr^{-1}) in the northeast of China (Yu et al., 2017). Each plot (6 m × 6 m) was surrounded by 2-m wide buffer strips. Purified sulfur fertilizer (elemental S > 99%) was mixed with 200 g soil collected from the untreated site nearby and applied by hand spreading annually. Sulfur powder in soil can be oxidized by soil microorganisms to form H^+ and SO_4^{2-} which can simulate soil acidification well (Duddigan et al., 2021). In present study, we collected plant and soil samples from 25 plots (five replicates) supplemented with five levels of S (0, 5, 10, 20, and 50 g S $m^{-2}yr^{-1}$).

2.2 Plant and soil sampling

On early August 2019, aboveground net primary productivity (ANPP) of plant communities was collected from peak aboveground plant biomass because all aboveground plant tissues would die during the winter. All living tissues were clipped within a randomly selected 1 m × 1 m quadrat in each plot, sorted to species and oven-dried at 65 °C for 48 h to measure peak species biomass and ANPP. The dried plant samples were prepared to measure leaf nutrients.

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We measured three aboveground morphological traits for two dominate species *Leymus chinensis* (*L. chinensis*) and *Carex duriuscula* (*C. duriuscula*). Ten plant individuals with complete shoot were randomly selected in each plot for each species. These plant individuals were measured for maximum natural height and then clipped at the ground level. All the samples immediately placed in a portable refrigerator and then detached to measure leaf area in laboratory. To guarantee water saturation of the leaves, the sampled leaves were immersed 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 area was scanned using a scanner (Eption Perfection V39, Seiko Epson Corporation, Japan) and then dried at 60 °C for 72 h to weigh for dry mass. Specific leaf area (SLA, $cm^2\ g^{-1}$) was calculated as the ratio of 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 cm (length) × 30 cm (width) × 30 cm (depth) soil block was collected using a steel plate and a shovel from each plot, resulting in a total of 25 soil blocks. Each harvested soil block was immediately transported to the processing area and then the soil blocks were gently loosened by hands to separate roots from soils. All separated plant roots were carefully washed to remove the adhering soil and stored in iceboxes to the laboratory. Before determining root morphological and chemical traits, all root samples were frozen at -20 °C. At least 10 intact individual plants of *L. chinensis* and *C. duriuscula* in each plot were used for determining root nutrient traits (root [N], [P], [S], [Ca], [Fe], and [Mn]) and root morphological traits. Total root length, surface area and volume were determined using the scanned images by the software of WinRHIZO (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 of the above samples were dried at 65 °C to constant mass for determining root biomass at species and community level. Root and leaf N concentrations were determined using

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an elemental analyzer (Vario EL III, Elementar, Hanau, Germany). Both root and leaf P, S, Ca, Fe and Mn concentrations were digested with 8 mL HNO_3 + 4 mL $HClO_4$ and then determined by inductively coupled plasma optical emission spectrometry (5100 ICP-OES; Perkin Elmer, America).

Soil sampling (0 - 10 cm depth) was performed using a soil auger (5 cm inner diameter). For each plot, three cores were combined into one homogeneous sample. After removing the visible plant detritus and rock, we sieved the fresh soils through a 2-mm screen and divided each soil sample into two subsamples. One subsample was immediately extracted with 2 mol L^{-1} KCl solution. The extracted solution was analyzed for nitrate (NO_3^-) and ammonium (NH_4^+) concentrations using an autoAnalyser III continuous Flow Analyzer (Bran and Luebbe, Norderstedt, Germany). The other subsample was air-dried for physicochemical properties determination. Soil pH was determined in 2.5: 1 (v/w) water/soil ratio with a digital pH meter (Precision and Scientific Instrument Co. Ltd., Shanghai, China). Soil available P concentration was extracted with 0.5 mol L^{-1} $NaHCO_3$ solution and soil available S concentration was extracted with 0.1 mol L^{-1} $Ca(H_2PO_4)_2$ (Tabatabai and Bremner, 1972) following absorbance measurement on a UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan) at 880 nm and 440 nm, respectively. Soil exchangeable Ca^{2+}, Al^{3+}, diethylene triamine pentaacetic acid (DTPA)-Fe and Mn were determined according to the methods used in our previous studies (Feng et al., 2019; Li et al., 2021).

2.3 Statistical analyses

The effects of S addition on soil properties, plant traits and biomass were analyzed using one-way analysis of variance (ANOVA) with Duncan test. Pearson's correlation analysis was used to explore the relationship between plant traits, plant biomass and soil abiotic variables across the S-addition levels. All these statistical analyses were performed using SPSS16.0 (SPSS Inc., Chicago, USA) with significance accepted at $p < 0.05$.

We used structural equation modelling (SEM) to analyze the direct and indirect effects of S addition meditating grassland plant aboveground and root biomass from the

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perspective of plant traits and soil factors. Prior to SEM analysis, the number of variables were reduced by conducting principal component analysis (PCA) on soil variables (pH, NH_4^+-N, NO_3^--N, available P, available S, exchangeable cations Ca^{2+} and Al^{3+}, DTPA-Fe and DTPA-Mn), aboveground morphological traits (Height, SLA, LDMC), leaf nutrient traits (Ca, Fe, Mn), root morphological traits (SRL, SRA, RTD) and root nutrient traits (N, P, S, Ca, Fe, Mn) of the two species (Chen et al., 2013). We then used the first principal components (PC1) for the subsequent SEM analysis to represent soil acidification (PC1 explained 94.8% of the variation), soil nutrients (PC1 explained 62.3% of the variation), root nutrient traits in *C. duriuscula* (PC1 explained 45.7% of the variation), aboveground morphological traits in *L. chinensis* (PC1 explained 54.7% of the variation) (Table S1). A conceptual model of hypothetical relationships was constructed (Fig. S1), assuming that S addition would directly impact aboveground and belowground traits and biomass, or indirectly through altering soil pH, soil nutrient availability, soil cations and plant traits. The SEM analyses were performed using IBM SPSS AMOS 24.0 and the PCA analyses were performed using the vegan package in R 4.2.2.

3 Results

3.1 Effects of S addition on soil properties

Sulfur addition significantly decreased soil pH from 6.95 to 5.19, but increased soil exchangeable Al concentration only in the highest S-addition level of 50 g S m^{-2} yr^{-1} (Table 1). Similarly, S addition increased soil ammonium concentration but decreased nitrate concentration in the highest S addition treatment compared to the control (Table 1). Soil available P, available S, DTPA-Fe and DTPA-Mn concentration increased with increasing S addition rate, while soil exchangeable Ca concentration decreased (Table 1).

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

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For the morphological traits, S addition enhanced plant height of *C. duriuscula*, but had no impact on *L. chinensis* (Fig. 1a). Sulfur addition significantly decreased SLA and increased LDMC of *L. chinensis*, whereas it had no effect on that of *C. duriuscula* (Fig. 1b and c). For the belowground tissues, S treatment increased SRL in two species, and only decreased SRA of *C. duriuscula* (Fig. 1d and e). However, RTD showed no significant change for the two species (Fig. 1f).

For the nutrient traits, S addition had no impact on leaf [N], [P], and [Ca], and increased leaf [S] and [Mn] of the two species, while decreased leaf [Fe] of *C. duriuscula* but increased leaf [Fe] of *L. chinensis* (Fig. 2). An increase in root [N], root [P], root [S] of *C. duriuscula* was found under S addition, but not for *L. chinensis* (Fig. 2h, i and j). Sulfur addition decreased root [Ca] of *C. duriuscula*, but had no impact on *L. chinensis* (Fig. 2k). Root [Fe] showed similar patterns with leaf [Fe] with a decrease in *C. duriuscula* and an increase in *L. chinensis* (Fig. 2l). Root [Mn] of species were enhanced by S addition (Fig. 2m).

3.3 Effects of S addition on above- and belowground biomass

In the third year, S addition suppressed aboveground biomass of plant community (Fig. 3). Aboveground biomass of the two dominant species showed contrasting responses to S addition, with an increase for *C. duriuscula* but a decrease for *L. chinensis* (Fig. 3). Moreover, S addition significantly increased belowground biomass of plant community owing to the increase in *C. duriuscula*, while it had no impact on the belowground biomass of *L. chinensis* (Fig. 3).

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

According to correlation analysis (Figs. S2 and S3), the aboveground morphological traits, leaf and the root nutrient traits showed species-specific responses. This was mainly due to the increase in soil acidity, Al^{3+} toxicity and nutrient imbalance (*i.e.*, the deficient or excessive of certain nutrients in the soil) induced by S addition, which fitted the structural equation modelling (SEM) well (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

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265 belowground biomass was mainly implemented through decreasing soil pH together
266 with the imbalance of soil available nutrients, altering the leaf and root nutrient traits,
267 and the belowground biomass and of *C. duriuscula*, which accounted for 69% of the
268 variation in community belowground biomass (Fig. 4). The indirect negative effect of
269 S addition on community aboveground biomass was mainly achieved through soil
270 acidification, the aboveground morphological traits and aboveground biomass of *L.*
271 *chinensis*, which accounted for 59% of the variation in community aboveground
272 biomass (Fig. 4).

273 ## 4. Discussion

274 ### 4.1 Species-specific trait responses to S addition

275 Trait response patterns was different between *L. chinensis* and *C. duriuscula* under S
276 addition. Specifically, nutrient traits of *L. chinensis* was less plastic, as evidenced by
277 unchanged concentrations of N, P, S, and Ca, comparing with *C. duriuscula*. Indeed, *L.*
278 *chinensis* was suggested to be a highly homoeostatic species with greater stability in
279 elemental composition in a temperate steppe (Yu et al., 2010). Higher macroelement
280 homeostasis helps plant maintain function and productivity stability to resist changes
281 in soil environment (Yu et al., 2010; Feng et al., 2019).

282 It was interesting to note that both leaf and root [Fe] in *L. chinensis* increased with
283 S addition and were not associated with soil available [Fe] (Figs. 2 and S2). Iron uptake
284 and assimilation had been shown to be dependent on sulfate availability (Zuchi et al.,
285 2012). Previous research demonstrated close relationships between Fe and S nutrition,
286 suggesting common regulatory mechanisms for the homeostasis of the two elements
287 (Forieri et al., 2013). For grasses, S addition could enhance assimilation of plant S and
288 subsequently incorporated into methionine in order to accelerate the secretion of
289 phytosiderophore (Zuchi et al., 2012; Courbet et al., 2019). However, Fe absorption of
290 *C. duriuscula* was inhibited by soil acidification which was consistent with Fe (III)-
291 reduction-based mechanism (Tian et al., 2016). Namely, acquisition of Fe by non-
292 graminaceous monocotyledonous species was mediated by the reduction of Fe^{3+} to Fe^{2+}
293 catalyzed by the ferric chelate reductase in root cells, and Fe^{2+} absorption can be further

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curbed by the 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. 2 and 5). Sulfur addition increased tissue [Mn] greater in *C. duriuscula* than in *L. chinensis*.

L. chinensis decreased SLA and increased LDMC to reduce the loss of water and nutrients, which showed conservative resource-uptake strategy under soil acidification stress. The variations in SLA and LDMC of *L. chinensis* were significantly correlated with soil exchangeable Al, implying that conservative traits might also link with Al-resistant strategy of species (Poozesh et al., 2007). As soil pH decreased, soil nitrate was reduced and positively correlated with SLA but negatively with LDMC of *L. chinensis* (Table 1 and Fig. S2). Soil nitrification had been shown to be positively related to leaf traits (such as leaf [N] and SLA) (Laughlin et al., 2011). This suggested that the decrease of soil nitrate under soil acidification could be an important driver of plant trait variation. For *L. chinensis*, belowground traits were insensitive to S addition as compared with *C. duriuscula*. One possible explanation for this insensitivity might be that deep-rooted species were much more resistant to changing soil environment than the shallow-rooted species (such as sedge *C. duriuscula*) (Zhang et al., 2019). We found both species invested more in enhancing SRL under soil acidification, which was in agreement with Göransson et al. (2011) that grass species increased root length to avoid acid stress. These results indicated 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, we explored the important role of morphological and nutrient traits in mediating aboveground and belowground biomass changes under S addition. We found that aboveground and root traits of two species exhibited contrasting adaptive strategies in acquiring aboveground and belowground resources which were associated with their biomass (Figs. 4 and 5). Importantly, SEM showed

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that the decrease in aboveground biomass of *L. chinensis* was related to the increased soil acidification and the conservative responses in aboveground morphological traits under S addition (Fig. 4). *L. chinensis* seemed to be a nitrophilic and resource-acquisitive species under N-rich environment (Feng et al., 2019; Yang et al., 2019), but it was at a disadvantage 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. S2). Soil acidification resulted in enhanced toxic effects of proton and exchangeable Al (Roem and Berndse, 2000). From environmental stress hypothesis perspective, the species could employ different strategies to mitigate such environmental stress which associated with trait responses (Encinas-Valero et al., 2022). Usually, SLA and LDMC were prominent indicators of plant strategy with respect to productivity as related to environmental stress and disturbance regimes. Stress tolerant species normally had lower growth rates, photosynthetic rates, and SLA but higher LDMC (Pérez-Harguindeguy et al., 2013). Sulfur addition induced acidity stress for plants, leading to reduced SLA accompanied with lower photosynthesis and decreased plant aboveground productivity.

Notably, *L. chinensis* played a dominate role in aboveground productivity which was consistent with the finding that grasses occupied a mean coverage of around 60% in acid grassland and Heathland (Tibbett et al., 2019). Therefore, the decreasing aboveground biomass of *L. chinensis* was one reason for the decline of community aboveground productivity. Our results provided compelling evidences that S-induced soil acidification could alter grassland aboveground biomass via modifying shoot morphological traits mediated by soil abiotic factors. 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). Our study for the first time revealed that plant leaf morphological traits might be an important regulatory factor for grassland productivity.

In contrast, sedge (*C. duriuscula*) was more tolerant than perennial rhizome grass (*L. chinensis*) under soil acidification. This was partly supported by similar results obtained in alpine and typical steppe grassland ecosystems (Chen et al., 2015; Wang et

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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 nutrient acquisition. SEM provided further evidence that for *C. duriuscula*, the higher nutrient demand (such as root [N], [P], [S], [Mn]) was matched by high root biomass investment under S treatment (Fig. 4). The increased root biomass of *C. duriuscula* promoted the increased belowground biomass of plant community which could be related to the shifts in soil available nutrients under S addition. Our present study provided direct evidence that *C. duriuscula* was considered to be a high nutrient-requiring species and thereby its biomass growth increased with soil acidification stress (Figs. 4 and 5). The findings suggested that the sedge played an important role in preventing grassland productivity decline in acidified soils.

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 long-term, however, asymmetric light competitive advantage of larger individuals (*L. chinensis*) rather than the competition of soil resources (DeMalach and Kadmon, 2017), will make a decisive effect on community productivity and diversity under soil acidification. Contrary to previous findings by Wang et al. (2020), who reported that diameter of 3rd-order roots contributed to the increase of community belowground biomass under soil acidification in an alpine grassland. Our study provided a novel insight that leaf and root nutrients as a whole jointly mediated community belowground biomass with soil acidification induced by S addition.

5 Conclusion

Our results highlighted that aboveground and root traits played important roles in mediating grassland plant competition for environment resources under soil acidification. Sulfur addition acidified soils, and lead to nutrient imbalance (higher ammonium, available P, Fe, Mn and exchangeable Al^{3+}, but lower nitrate and exchangeable Ca^{2+}). The dominate species *L. chinensis* showed conservative strategy,

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with decreased SLA and increased LDMC in response to S addition. Moreover, conservative traits were linked with stable root biomass but lower aboveground biomass as a direct impact from soil acidification. Conversely, *C. duriuscula* displayed acquisitive strategy, with increased shoot height and root traits ([N], [P], [S], [Mn], SRL) promoting both aboveground and root biomass under S addition, as mediated via altered soil acidity and nutrient availability. Such divergent and species-specific responses was strongly driven by soil environmental conditions which resulted in inconsistent responses of grassland community aboveground and belowground biomass to S addition. As continuous S deposition causes widespread acidification and soil functional degradation problems across the world, our results implied the important roles of both aboveground and root traits in regulating species and community biomass under soil acidification.

Author contributions. All authors contributed to the design of the study. TL and HL conducted the experimental work and the data analysis. XF wrote the manuscript with RW, JC and YJ.

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

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Data availability. Data will be made available on request from the corresponding author.

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Figures

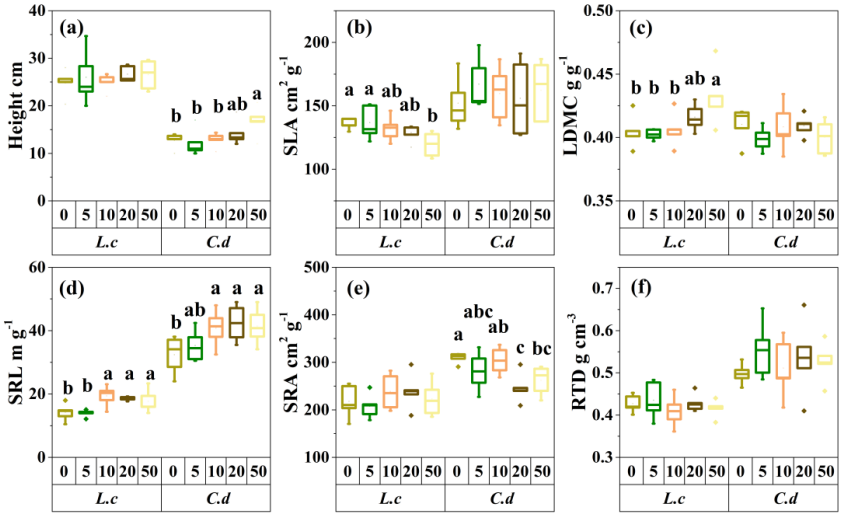

Fig. 1 The response of the morphological traits to S addition for the two dominate species in a meadow steppe. Abbreviations: SLA, Specific leaf area; LDMC, Leaf dry matter content; SRL, specific root length; SRA, specific root area; RTD, root tissue density; *L.c*, *L. chinensis*; *C.d*, *C. duriuscula*. 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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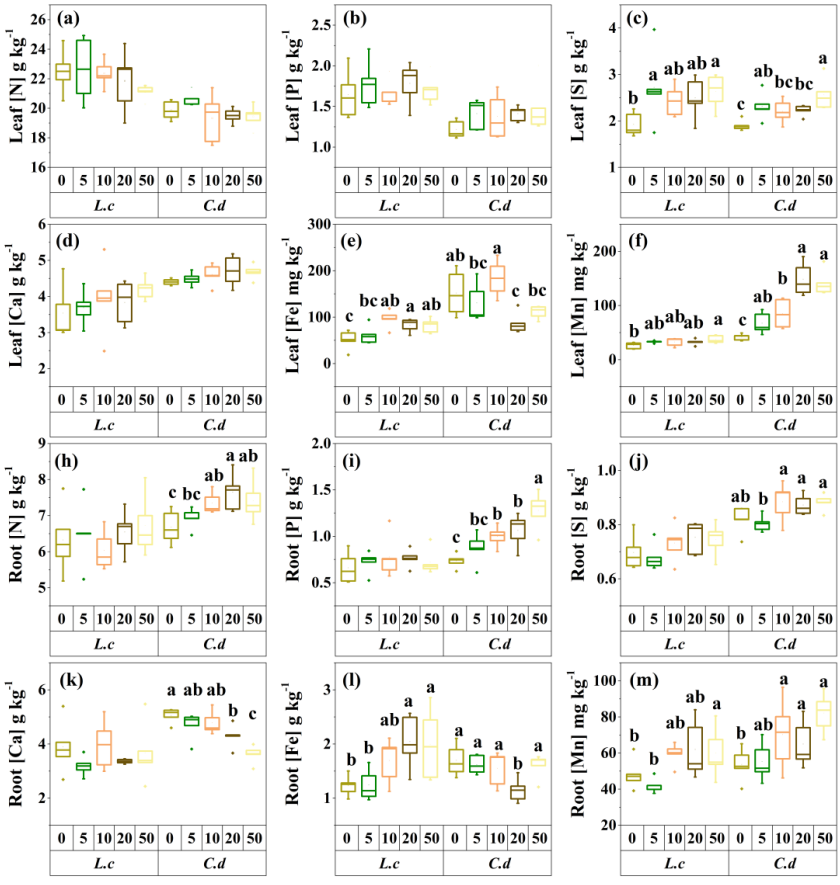

Fig. 2 The response of the chemical traits to S addition for the two dominate species in a meadow steppe. Abbreviations: Leaf [N], leaf N concentration; Leaf [P], leaf P concentration; Leaf [S], leaf S concentration; Leaf [Ca], leaf Ca concentration; Leaf [Fe], leaf Fe concentration; Leaf [Mn], leaf 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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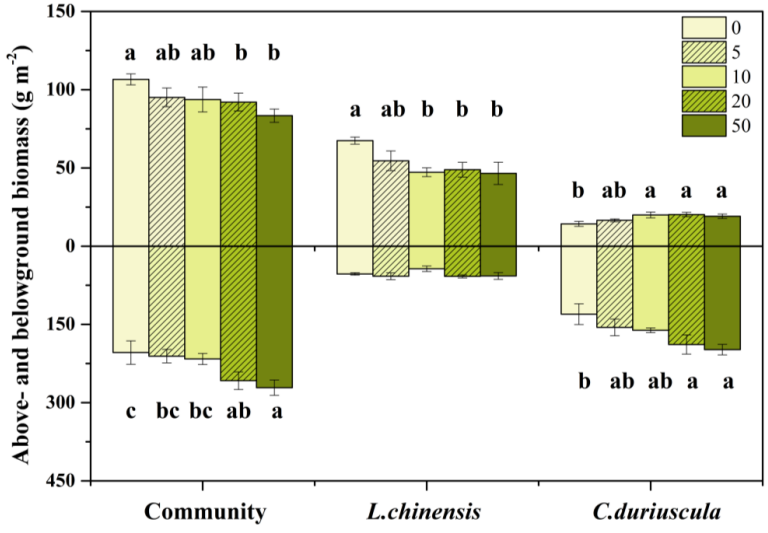

Fig. 3 Effects of S addition on community and species aboveground and belowground biomass. Bars are means ± the standard error. Lower case letters indicate significant difference among treatments ($P < 0.05$).

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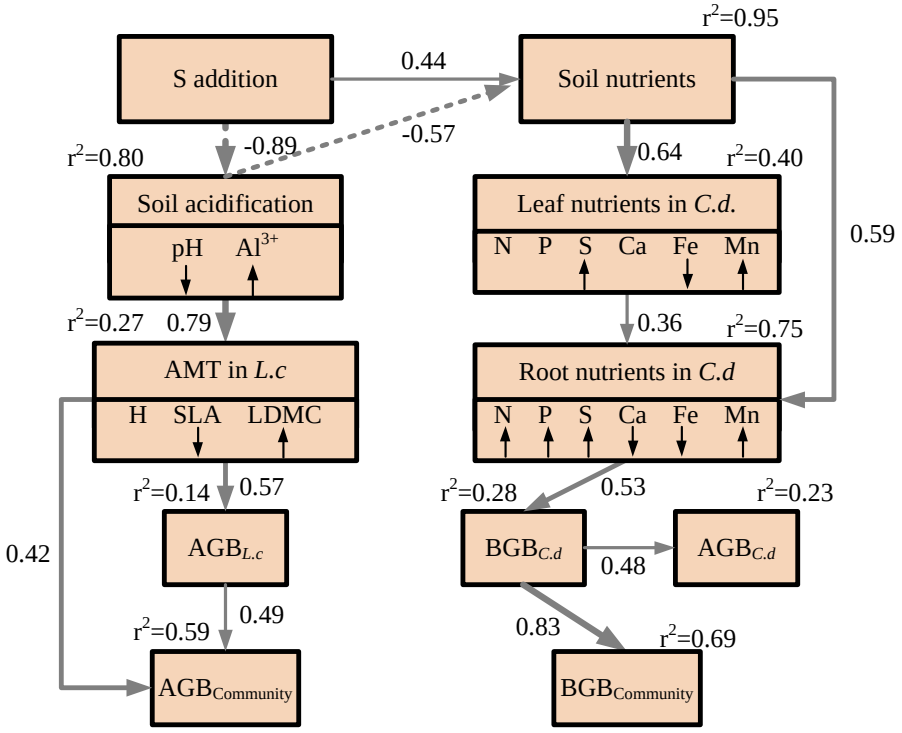

Fig. 4 Structural equation model of S addition on plant community biomass through the plausible pathways. Square boxes indicate the included variables in the analysis: S addition; Soil nutrients include soil NH_4^+-N and NO_3^--N concentrations, soil available phosphorus, soil available sulfur; soil 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: χ^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.

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672 Solid and dashed arrows represent significant positive and negative pathways ($P < 0.05$),
673 respectively. Nonsignificant ($P > 0.05$) pathways are not shown. Values at each arrow
674 indicate the standard path coefficient, which is equivalent to the correlation coefficient.
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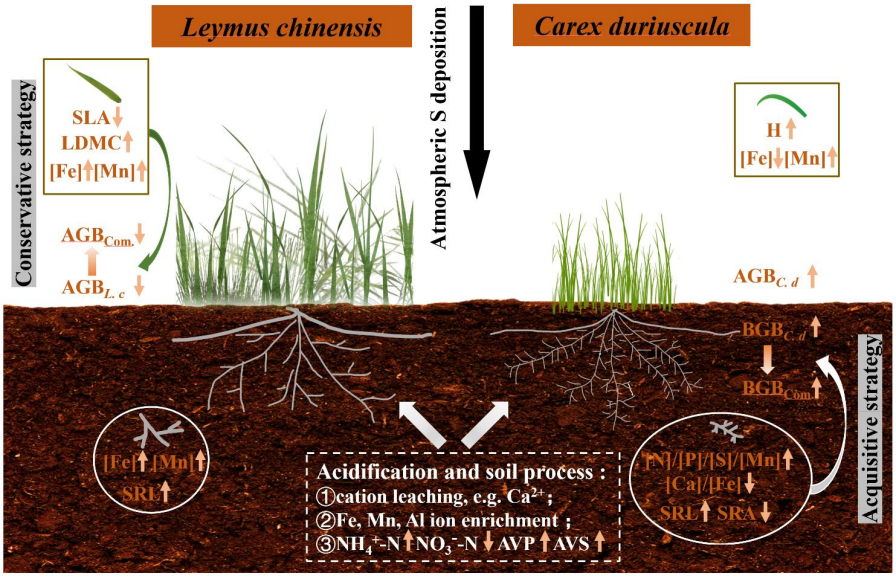

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678 **Fig. 5** Schematic diagram illustrating the ecological effects of S-induced soil
679 acidification on above- and belowground biomass and traits of two dominate species in
680 a meadow steppe. ↑ = increase in response to S addition; ↓ = decrease in response to S
681 addition; Com. = Community; AVP = Soil available phosphorus; AVS = Soil available
682 sulfur.
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Table

Table 1 Effects of S addition on soil abiotic variables. All numbers refer to the mean (the standard error). Lower case letters indicate significant difference among treatments ($P < 0.05$).

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

Note: Ex. Al^{3+}: Exchangeable Al^{3+}, mg kg^{-1}; Ammonium: soil NH_4^+-N concentration, mg kg^{-1}; Nitrate: soil NO_3^--N concentration, mg kg^{-1}; AVP: soil available phosphorus, mg kg^{-1}; AVS: soil available sulfur, mg kg^{-1}; DTPA-Fe: Soil DTPA-Fe concentration, mg kg^{-1}; DTPA-Mn: Soil DTPA-Mn concentration, mg kg^{-1}; Ex. Ca: Exchangeable Ca^{2+}, cmol kg^{-1}.