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

Xue Feng [1], Ruzhen Wang [1,2], Tianpeng Li [1], Jiangping Cai [1], Heyong Liu [1,2], Hui Li [1], Yong Jiang [1,2,*]

[1]Erguna Forest-Steppe Ecotone Ecosystem Research Station, Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

[2]College of Life Sciences, Hebei University, Baoding 071002, Hebei, China

* Correspondence: Yong Jiang (jiangyong@iae.ac.cn).

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% 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

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 a significant decline in mean soil pH from 7.84 to 7.21 during 1980s- 2000s, 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., 2013a; Tibbett et al., 2019; Duddigan et al., 2021).

In natural ecosystems, sulfur is an essential nutrient in forming plant proteins because it is a constituent of certain amino acids, but S limitation rarely occurs (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., 2005), but promotes 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 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., 2013). 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 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., 2022). Grass species may also increase root length to avoid acid and Al^{3+} stresses (Göransson et al., 2011). In general, species with acquisitive strategy accumulate greater biomass in a rapid way, but species 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. Some plants can cope with nutrient deficiency in acidic soils via modifications to their root morphologies and in their nutrient uptakes and metabolisms (Hammond et al., 2004). Plants growing in resource-poor soils tend to have lower specific root length (SRL) and lower root nutrient concentrations for the conservation of resources (Delpiano et al., 2020). 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). But in natural ecosystems, grasses develop densely branched root systems with higher nutrient use efficiency are more stress-tolerant to nutrient deficiency to maintain nutrient balance and growth (Tian et al., 2022). 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 functional traits in both above- and belowground components of different species respond to soil acidification and their linkages with 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. The perennial rhizome grass, a taller *L. chinensis* is widely distributed in arid and semi-arid areas of northern China. This species occupies the top layers of the studied grassland communities, likely giving it an advantage in resource acquisition, especially in terms of light. Additionally, grasses generally exhibit flexibility in absorbing various soil N forms, thereby expanding their ecological niche (Grassein et al., 2015). The perennial rhizomatous sedge, a shorter subordinate species *C. duriuscula*, indicator plant for soil degradation, possesses cluster root, tends to consume more photosynthetic products to acquire nutrients (Zhang et al., 2021). Moreover, 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). 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 soil acidification caused by S addition would lead to a nutrient imbalance in grassland soil. Grass *L. chinensis* may respond to soil acidification by adapting its aboveground light acquisition traits to maintain plant biomass. However, in acidified soil, sedge *C. duriuscula* may employ the increased tissue nutrient concentrations as a strategy to improve its acid resistance, which subsequently leads 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 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*.

The experimental field was a natural steppe, which had been mown annually for forage harvest until 2013, and fenced to exclude livestock grazing since then. A field elemental S addition experiment was established in 2017 to simulate soil acidification caused by atmospheric S deposition in a homogeneous and flat field containing naturally assembled communities. The vegetation in the experimental plots is composed of the dominant species (relative abundance >40 %) *Leymus chinensis*, subordinate species (relative abundance between 1 % and 30 %), including *Stipa baicalensis, Carex duriuscula*, *Cleistogenes squarrosa*, *Achnatherum sibiricum*, *Cymbaria dahurica*, *Pulsatilla turczaninovii*, *Thermopsis lanceolala* and *Achnatherum sibiricum*. 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 (Fig. S2). 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 in late May since 2017. 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

In 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 by 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.

We measured three aboveground morphological traits for two dominant 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 were 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 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).

Fresh 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. Then a 10 g of fresh soil 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 aluminum (Al^{3+}) concentration was measured using titration by 0.25 M NaOH to pH 7.0 after extraction with 1 M KCl solution from air-dried soil samples. Soil exchangeable calcium (Ca^{2+}) was extracted by 1 M NH_4OAc (pH = 7.0) at a 1:10 ratio (w/v) for 30 min. Diethylene triamine pentaacetic acid (DTPA)-Fe and Mn were extracted from 10 g of air-dried soil sample with 20 ml of 0.005 M diethylenetriamine pentaacetic acid (DTPA), 0.01 M $CaCl_2$, and 0.1 M triethanolamine (TEA) at pH 7.3 and determined using an atomic absorption spectrophotometer (AAS, Shimadzu, Japan) (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 indirect effects of S addition meditating grassland plant aboveground and root biomass from the 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. 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 soil physicochemical properties, and indirectly influence aboveground and belowground biomass through altering soil pH, soil nutrient availability and plant traits. The SEM analyses were performed using AMOS 24.0 (Amos Development Co., Maine, USA) 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 biomass

In the third year, S addition suppressed aboveground biomass of plant community (Fig. 1). 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. 1). 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. 1).

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

For the morphological traits, S addition enhanced plant height of *C. duriuscula*, but had no impact on *L. chinensis* (Fig. 2a). Sulfur addition significantly decreased SLA and increased LDMC of *L. chinensis*, whereas it had no effect on that of *C. duriuscula* (Fig. 2b and c). For the belowground tissues, S treatment increased SRL in two species, and only decreased SRA of *C. duriuscula* (Fig. 2d and e). However, RTD showed no significant change for the two species (Fig. 2f).

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. 3). An increase in root [N], root [P], root [S] of *C. duriuscula* was found under S addition, but not for *L. chinensis* (Fig. 3h, i and j). Sulfur addition decreased root [Ca] of *C. duriuscula*, but had no impact on *L. chinensis* (Fig. 3k). Root [Fe] showed similar patterns with leaf [Fe] with a decrease in *C. duriuscula* and an increase in *L. chinensis* (Fig. 3l). Root [Mn] of species were enhanced by S addition (Fig. 3m).

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

According to correlation analysis (Figs. S3 and S4), 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 belowground biomass was mainly implemented through decreasing soil pH together with the imbalance of soil available nutrients, altering the leaf and root nutrient traits, and the belowground biomass and of *C. duriuscula*, which accounted for 69% of the variation in community belowground biomass (Fig. 4). The indirect negative effect of S addition on community aboveground biomass was mainly achieved through soil acidification, the aboveground morphological traits and aboveground biomass of *L. chinensis*, which accounted for 59% of the variation in community aboveground biomass (Fig. 4).

4. Discussion

4.1 Species-specific trait responses to S addition

Trait response patterns was different between *L. chinensis* and *C. duriuscula* under S addition. Specifically, nutrient traits of *L. chinensis* were less plastic, as evidenced by unchanged concentrations of N, P, S, and Ca, comparing with *C. duriuscula*. Indeed, *L. 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 function and productivity stability to resist changes in soil environment (Yu et al., 2010; Feng et al., 2019).

It was interesting to note that both leaf and root [Fe] in *L. chinensis* increased with S addition and were not associated with soil available [Fe] (Figs. 3 and S3). Iron uptake and assimilation had 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 and subsequently incorporated into methionine in order to accelerate the secretion of phytosiderophore (Zuchi et al., 2012; Courbet et al., 2019). However, Fe absorption of *C. duriuscula* was inhibited by soil acidification which was consistent with Fe (III)-

reduction-based mechanism (Tian et al., 2016). Namely, acquisition of Fe by non-graminaceous monocotyledonous species was mediated by the reduction of Fe^{3+} to Fe^{2+} catalyzed by the ferric chelate reductase in root cells, and Fe^{2+} absorption can be further 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. 3 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. S3). 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 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 (Figs. 4 and 5). *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. S3). 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. Damages in photosynthetic function accompanied by oxidative stress were found in woody tree species under the threat of acid rain (Chen et al., 2013b), it is still less understanding to physiological and biochemical responses of different functional groups to soil acidification in grassland ecosystems. The future researches about plant photosynthetic and antioxidant responses by soil acidification are critically needed to test.

We found that plant community aboveground biomass exhibited a tendency to decline from 22% to 11% under soil acidification, although the overall effect was rather weak between pH 6.7 and pH 5.19 (Fig. 1, Table 1). Notably, *L. chinensis* played a dominant 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 (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, our study contributes to a deeper understanding that leaf morphological traits of dominant species play a crucial role in regulating grassland productivity in response to soil acidification.

In contrast, belowground biomass of *C. duriuscula* and plant community both significantly increased (ranging from 19 to 52%) with soil acidification (Fig. 1). 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 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). Our short-term findings suggest that the sedge play an important role in preventing the decline of grassland productivity in acidified soils, reflecting transient dynamics. Consistent with results from a long-term acidification experiment (Tibbett et al., 2019), compensatory growth of acid-tolerant species is probably key to maintain grassland productivity over the long 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 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 3^{rd}-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 dominant species *L. chinensis* showed conservative strategy, 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.

References

Bolan, N. S., Adriano, D. C., and Curtin, D.: Soil acidification and liming interactions with nutrient and heavy metal transformation and bioavailability, Adv. Agron., 78, 5-272, https://doi.org/10.1016/S0065-2113(02)78006-1, 2003.

Bontti, E. E., Decant, J. P., Munson, S. M., Gathany, M. A., Przeszlowska, A., Haddix, M. L., Owens, S., Burke, I. C., Parton, W. J., and Harmon, M. E.: Litter decomposition in grasslands of central North America (US Great Plains), Global Chang Biol., 15, 1356-1363. https://doi.org/ 10.1111/j.1365-2486.2008.01815.x, 2009.

Chen, D., Lan, Z., Bai, X., Grace, J. B., and Bai, Y.: Evidence that acidification-induced declines in plant diversity and productivity are mediated by changes in below-ground communities and soil properties in a semi-arid steppe, J. Ecol., 101, 1322-1334, https://doi.org/10.1111/1365-2745.12119, 2013a.

Chen, D., Wang, Y., Lan, Z., Li, J., Xing, W., Hu, S. and Bai, Y.: Biotic community shifts explain the contrasting responses of microbial and root respiration to experimental soil acidification, , 90, 139-147, https://doi.org/10.1016/j.soilbio.2015.08.009, 2015.

Chen, J., Wang, W. H., Liu, T., Wu, F., and Zheng, H.: Photosynthetic and antioxidant responses of *Liquidambar formosana* and *Schima superba* seedlings to sulfuric-rich and nitric-rich simulated acid rain. Plant Physiol. Bioch., 64, 41-51, https://doi.org/10.1016/j.plaphy.2012.12.012, 2013b.

Clark, C. M., Simkin, S. M., Allen, E. B., Bowman, W. D., Belnap, J., Brooks, M. L., Collins, S. L., Geiser, L. H., Gilliam, F, S., Jovan, F. S., Pardo, L. H., Schulz, B. K., Stevens, C. J., Suding, K. N., Throop, H. L., and Waller, D. M.: Potential vulnerability of 348 herbaceous species to atmospheric deposition of nitrogen and sulfur in the United States, Nat. Plants, 5, 697-705, https://doi.org/10.1038/s41477-019-0442-8, 2019.

Cliquet, J. B. and Lemauviel-Lavenant, S.: Grassland species are more efficient in acquisition of S from the atmosphere when pedospheric S availability decreases, Plant Soil, 435, 69-80, https://doi.org/10.1007/s11104-018-3872-6, 2019.

Courbet, G., Gallardo, K., Vigani, G., Brunel-Muguet, S., Trouverie, J., Salon, C., and Ourry, A.: Disentangling the complexity and diversity of crosstalk between sulfur and other mineral nutrients in cultivated plants, J. Exp. Bot., 70, 4183-4196, https://doi.org/10.1093/jxb/erz214, 2019.

Curie, C. and Briat, J. F.: Iron transport and signaling in plants, Annu. Rev. Plant Biol., 54, 183-206, https://doi.org/10.1146/annurev.arplant.54.031902.135018, 2003.

De Battisti, D., Fowler, M. S., Jenkins, S. R., Skov, M. W., Bouma, T. J., Neyland, P. J., and Griffin, J. N.: Multiple trait dimensions mediate stress gradient effects on plant biomass allocation, with implications for coastal ecosystem services, J. Ecol., 108, 1227-1240, https://doi.org/10.1111/1365-2745.13393, 2020.

Delpiano, C.A., Prieto, I., Loayza, A.P., Carvajal, D.E., and Squeo, F.A.: Different responses of leaf and root traits to changes in soil nutrient availability do not converge into a community-level plant economics spectrum. Plant Soil 450, 463-478, https://doi.org/10.1007/s11104-020-04515-2, 2020.

DeMalach, N. and Kadmon, R.: Light competition explains diversity decline better than niche dimensionality, Funct. Ecol., 31, 1834-1838, https://doi.org/10.1111/1365-2435.12841, 2017.

Duan, L., Yu, Q., Zhang, Q., Wang, Z., Pan, Y., Larssen, T., Tang, J., and Mulder, J.: Acid deposition in Asia: Emissions, deposition, and ecosystem effects, Atmospheric Environ., 146, 55-69, http://doi.org/10.1016/j.atmosenv.2016.07.018, 2016.

Duddigan, S., Fraser, T., Green, I., Diaz, A., Sizmur, T., and Tibbett, M.: Plant, soil and faunal responses to a contrived pH gradient, Plant Soil, 462, 505-524, https://doi. org/10.1007/s11104-021-04879-z, 2021.

Encinas-Valero, M., Esteban, R., Hereş, A. M., Vivas, M., Fakhet, D., Aranjuelo, I., Solla, A., Moreno, G., and Curiel Yuste, J.: Holm oak decline is determined by shifts in fine root phenotypic plasticity in response to belowground stress, New Phytol., 235, 2237-2251, https://doi.org/10.1111/nph.18182, 2022.

Feng, X., Wang, R., Yu, Q., Cao, Y., Zhang, Y., Yang, L., Dijkstra, F. A., and Jiang, Y.: Decoupling of plant and soil metal nutrients as affected by nitrogen addition in a meadow steppe, Plant Soil, 443, 337-351, https://doi.org/10.1007/s11104-019 -04217-4, 2019.

Forieri, I., Wirtz, M., and Hell, R.: Toward new perspectives on the interaction of iron and sulfur metabolism in plants, Front. Plant Sci., 4, 357, https://doi.org/10.3389/fpls.2013.00357, 2013.

Garrison, M. T., Moore, J. A., Shaw, T. M., and Mika, P. G.: Foliar nutrient and tree growth response of mixed-conifer stands to three fertilization treatments in northeast Oregon and north central Washington, For. Ecol. Manag., 132, 183-198, https://doi.org/10.1016/S0378-1127(99)00228-5, 2000.

Geng, Y., Wang, L., Jin, D., Liu, H., and He, J.: Alpine climate alters the relationships between leaf and root morphological traits but not chemical traits, Oecologia, 175, 445-455, https://doi.org/10.1007/s00442-014-2919-5, 2014.

Göransson, P., Falkengren-Grerup, U., and Andersson, S.: *Deschampsia cespitosa* and soil acidification: general and trait-specific responses to acid and aluminium stress in a solution experiment, Nord. J. Bot., 29, 97-104, https://doi.org/10.1111/j.1756-1051.2010.00793.x, 2011.

Grassein, F., Lemauviel- Lavenant, S., Lavorel, S., Bahn, M., Bardgett, R. D., Desclos- Theveniau, M., and Laîné, P.: Relationships between functional traits and inorganic nitrogen acquisition among eight contrasting European grass species. Ann. Bot., 115, 107-115, https://doi.org/10.1093/aob/mcu233, 2015.

Gusewell, S.: N: P ratios in terrestrial plants: Variation and functional significance. New Phytol., 164, 243-266, https://doi.org/10.1111/j.1469-8137.2004.01192.x, 2004.

Haling, R. E., Richardson, A. E., Culvenor, R. A., Lambers, H., and Simpson, R. J.: Root morphology, root-hair development and rhizosheath formation on perennial grass seedlings is influenced by soil acidity, Plant Soil, 335, 457-468, https://doi.org/10.1007/s11104-010-0433-z, 2010.

Hammond, J.P., Broadley, M. R., and White, P. J.: Genetic responses to phosphorus deficiency. Ann. Bot., 94, 323-332, https://doi.org/10.1093/aob/mch156, 2004.

Hao, M., Messier, C., Geng, Y., Zhang, C., Zhao, X., and von Gadow, K.: Functional traits influence biomass and productivity through multiple mechanisms in a temperate secondary forest, Eur. J. For. Res., 139, 959-968, https://doi.org/10.1007/s10342-020-01298-0, 2020.

Jaggi, R. C., Aulakh, M. S., and Sharma, R.: Impacts of elemental S applied under various temperature and moisture regimes on pH and available P in acidic, neutral and alkaline soils, Biol. Fert. Soils, 41, 52-58, https://doi.org/10.1007/s00374 -004-0792-9, 2005.

Jung, K., Kwak, J. H, Gilliam, F. S., and Chang, S. X.: Simulated N and S deposition affected soil chemistry and understory plant communities in a boreal forest in western Canada. J. Plant Ecol., 11, 511-523, https://doi.org/10.1093/jpe/rtx030, 2018.

Kandlikar, G. S., Kleinhesselink, A. R., and Kraft, N. J.: Functional traits predict species responses to environmental variation in a California grassland annual plant community, J. Ecol., 110, 833-844, https://doi.org/10.1111/1365-2745.13845, 2022.

Kemmitt, S. J., Wright, D., and Jones, D. L.: Soil acidification used as a management strategy to reduce nitrate losses from agricultural land, Soil Biol. Biochem., 37, 867-875, https://doi.org/10.1016/j.soilbio.2004.10.001, 2005.

Laughlin, D.C.: Nitrification is linked to dominant leaf traits rather than functional diversity, J. Ecol., 99, 1091-1099, https://doi.org/10.1111/j.1365-2745.2011.01856.x, 2011.

Leifeld, J., Bassin, S., Conen, F., Hajdas, I., Egli, M., and Fuhrer, J.: Control of soil pH on turnover of belowground organic matter in subalpine grassland, Biogeochemistry, 112, 59-69, https://doi.org/10.1007/s10533-011-9689-5, 2013.

Li, T., Wang, R., Cai, J., Meng, Y., Wang, Z., Feng, X., Liu, H., Turco, R. F., and Jiang, Y.: Enhanced carbon acquisition and use efficiency alleviate microbial carbon relative to nitrogen limitation under soil acidification, Ecol. Process, 10, 1-13, https://doi.org/10.1186/s13717-021-00309-1, 2021.

Li, Y., Sun, J., Tian, D., Wang, J., Ha, D., Qu, Y., Jing, G., and Niu, S.: Soil acid cations induced reduction in soil respiration under nitrogen enrichment and soil acidification, Sci. Total Environ., 615, 1535-1546, http://doi.org/10.1016/j.scitotenv.2017.09.131, 2018.

Meng, C., Tian, D., Zeng, H., Li, Z., Yi, C., and Niu, S.: Global soil acidification impacts on belowground processes, Environ. Res. Lett., 14, 074003, https://doi.org/10.1088/1748-9326/ab239c, 2019.

Mitchell, R. J., Hewison, R. L., Fielding, D. A., Fisher, J. M., Gilbert, D. J., Hurskainen, S., Pakeman, R.J., Potts, J. M., and Riach, D.: Decline in atmospheric sulphur deposition and changes in climate are the major drivers of long-term change in grassland plant communities in Scotland, Environ. Pollut., 235, 956-964, https:// doi.org/10.1016/j.envpol.2017.12.086, 2018.

Mueller, K. E., Eissenstat, D. M., Hobbie, S. E., Oleksyn, J., Jagodzinski, A. M., Reich, P. B., Chadwick, O. A., and Chorover. J.: Tree species effects on coupled cycles of carbon, nitrogen, and acidity in mineral soils at a common garden experiment, Biogeochemistry, 111, 601-614, https://doi.org/10.1007/s10533-011-9695-7, 2012.

Pérez-Harguindeguy, N., Diaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M. S, Cornwell, W. K., Craine, J. M., Gurvich, D. E., Urcelay, C., Veneklaas, E. J., Reich, P. B., Poorter, L., Wright, I. J., Ray, P., Enrico, L., Pausas, J. G., de Vos, A. C., Buchmann, N., Funes, G., Quetier, F., Hodgson, J. G., Thompson, K., Morgan, H. D., ter Steege, H., van der Heijden, M. G. A., Sack, L., Blonder, B., Poschlod, P., Vaieretti, M. V., Conti, G., Staver, A. C., Aquino, S., and Cornelissen, J. H. C.: New handbook for standardised measurement of plant functional traits worldwide, Aust. Bot., 61, 167-234, http://doi.org/10.1071/BT 12225, 2013.

Pittman, J. K.: Managing the manganese: molecular mechanisms of manganese transport and homeostasis, New Phytol., 167, 733-742, http://doi.org/10.1111/j.1469-8137.2005.01453.x, 2005.

Poozesh, V., Cruz, P., Choler, P., and Bertoni, G., Relationship between the Al resistance of grasses and their adaptation to an infertile habitat, Ann. Bot., 99, 947-954, https://doi.org/10.1093/aob/mcm046, 2007.

Rabêlo, F. H. S., Lux, A., Rossi, M. L., Martinelli, A. P., Cuypers, A., and Lavres, J. Adequate S supply reduces the damage of high Cd exposure in roots and increases N, S and Mn uptake by Massai grass grown in hydroponics, Environ. Exp. Bot., 148, 35-46, https://doi.org/10.1016/j.envexpbot.2018.01.005, 2018.

Reich, P. B.: The world-wide ‘fast-slow’ plant economics spectrum: a traits manifesto, J. Ecol., 102, 275-301, https://doi.org/10.1111/1365-2745.12211, 2014.

Roem, W. J. and Berendse, F.: Soil acidity and nutrient supply ratio as possible factors determining changes in plant species diversity in grassland and heathland communities, Biol. Conserv., 92, 151-161, https://doi.org/10.1016/S0006-3207(99)00049-X, 2000.

Stevens, C. J., Thompson, K., Grime, J. P., Long, C. J., and Gowing, D. J.:

Contribution of acidification and eutrophication to declines in species richness of calcifuge grasslands along a gradient of atmospheric nitrogen deposition, Funct. Ecol., 24, 478-484, https://doi.org/10.1111/j.1365-2435.2009.01663.x, 2010.

Thomaes, A., De Keersmaeker, L., De Schrijver, A., Baeten, L., Vandekerkhove, K., Verstraeten, G., and Verheye, K.: Can soil acidity and light help to explain tree species effects on forest herb layer performance in post-agricultural forests?, Plant Soil, 373, 183-199, https://doi.org/10.1007/s11104-013-1786-x, 2013.

Tian, D. and Niu, S.: A global analysis of soil acidification caused by nitrogen addition, Environ. Res. Lett., 10, 024019, https://doi.org/10.1088/1748-9326/10/2/024019, 2015.

Tian, Q., Liu, N., Bai, W., Li, L., Chen. J., Reich, P. B., Yu Q., Guo, D., Smith, M. D., Knapp, A. K., Cheng, W., Lu, P., Gao, Y., Yang, A., Wang, T., Li, X, Wang, Z., Ma, Y., and Zhang, W.: A novel soil manganese mechanism drives plant species loss with increased nitrogen deposition in a temperate steppe, Ecology, 97, 65-74, https:// doi.org/10.1890/15-0917.1, 2016.

Tian, Q., Lu, P., Ma, P., Zhou, H., Yang, M., Zhai, X. Chen M., Wang, H., Li W., Bai, W., Lambers, H., and Zhang, W.: Processes at the soil-root interface determine the different responses of nutrient limitation and metal toxicity in forbs and grasses to nitrogen enrichment, J. Ecol., 109, 927-938, https://doi.org/10.1111/1365- 2745.13519, 2021.

Tian, Q., Lu, P., Zhai, X., Zhang, R., Zheng, Y., Wang, H., Nie, B., Bai, W., Niu, S., Shi, P., Yang, Y., Yang, D., Stevens, C., Lambers, H., and Zhang, W.: An integrated belowground trait‐based understanding of nitrogen‐driven plant diversity loss. Global Change Biol., 28, 3651-3664, https://doi.org/10.1111/ gcb.16147, 2022.

Tibbett, M., Gil-Martínez, M., Fraser, T., Green, I. D., Duddigan, S., De Oliveira, V. H., Raulund-Rasmussen, K., Sizmur, T. and Diaz, A.: Long-term acidification of pH neutral grasslands affects soil biodiversity, fertility and function in a heathland restoration, Catena, 180, 401-415, https://doi.org/10.1016/ j.catena.2019.03.013, 2019.

van Dobben, H. and de Vries, W.: Relation between forest vegetation, atmospheric deposition and site conditions at regional and European scales, Environ. Pollut., 158, 921-33, https://doi.org/10.1016/j.envpol.2009.09.015, 2010.

Violle, C., Navas, M. L., Vile, D., Kazakou, E., Fortunel, C., Hummel, I., and Garnier, E.: Let the concept of trait be functional!, Oikos, 116, 882-892, https://doi.org /10.1111/j.0030-1299.2007.15559.x, 2007.

Vitousek, P. M. and Howarth, R. W.: Nitrogen limitation on land and in the sea-how can it occur?, Biogeochemistry, 13, 87-115, https://doi.org/10.1007/BF00002772, 1991.

Wang, P., Guo, J., Xu, X., Yan, X., Zhang, K., Qiu, Y., Zhao, Q., Huang, K., Luo, X., Yang, F., Guo, H., and Hu, S.: Soil acidification alters root morphology, increases root biomass but reduces root decomposition in an alpine grassland, Environ. Pollut., 265, 115016, https://doi.org/10.1016/j.envpol.2020.115016, 2020.

Yang, F., Zhang, Z., Barberán, A., Yang, Y., Hu, S., Guo, H. Nitrogen-induced acidification plays a vital role driving ecosystem functions: Insights from a 6-year nitrogen enrichment experiment in a Tibetan alpine meadow, Soil Biol. Biochem., 153, 108107, https://doi.org/10.1016/j.soilbio.2020.108107, 2021.

Yang, G., Lü, X., Stevens, C. J., Zhang, G., Wang, H., Wang, Z., Zhang, Z., Liu, Z., and Han, X.: Mowing mitigates the negative impacts of N addition on plant species diversity, Oecologia, 189, 769-779, https://doi.org/10.1007/s00442-019-04353-9, 2019.

Yang, Y., Ji, C., Ma, W., Wang, S., Wang, S., Han, W., Mohammat, A., Robinson, D., and Smith, P.: Significant soil acidification across northern China's grasslands during 1980s-2000s, Global Change Biol., 18, 2292-2300, https://doi.org/10.1111 /j.1365-2486.2012.02694.x, 2012.

Yu, H., He, N., Wang, Q., Zhu, J., Gao, Y., Zhang, Y., Jia, Y., and Yu, G.: Development of atmospheric acid deposition in China from the 1990s to the 2010s, Environ. Pollut., 231, 182-190, http://doi.org/10.1016/j.envpol.2017.08.014, 2017.

Yu, Q., Chen, Q., Elser, J. J., He, N., Wu, H., Zhang, G., Wu, J., Bai, Y., and Han, X.: Linking stoichiometric homoeostasis with ecosystem structure, functioning and stability, Ecol. Lett., 13, 1390-1399, https://doi.org/10.1111/j.1461-0248.2010.01532.x, 2010.

Zhang, B., Cadotte, M. W., Chen, S., Tan, X., You, C., Ren, T., Chen, M., Wang, S., Li, W., Chu, C., Jiang, L., Bai, Y., Huang, J., and Han, X.: Plants alter their vertical root distribution rather than biomass allocation in response to changing precipitation, Ecology, 100, e02828, https://doi.org/10.1002 /ecy.2828, 2019.

Zhang, D., Peng, Y., Li, F., Yang, G., Wang, J., Yu, J., Zhou, G. and Yang, Y.: Above- and belowground resource acquisition strategies determine plant species responses to nitrogen enrichment. Ann. Bot., 128, 31-44, https://doi.org/10.1093 /aob/mcab032, 2021.

Zuchi, S., Cesco S., and Astolfi S.: High S supply improves Fe accumulation in durum wheat plants grown under Fe limitation, Environ. Exp. Bot., 77, 25-32, https://doi. org/10.1016/j.envexpbot.2011.11.001, 2012.

Figures

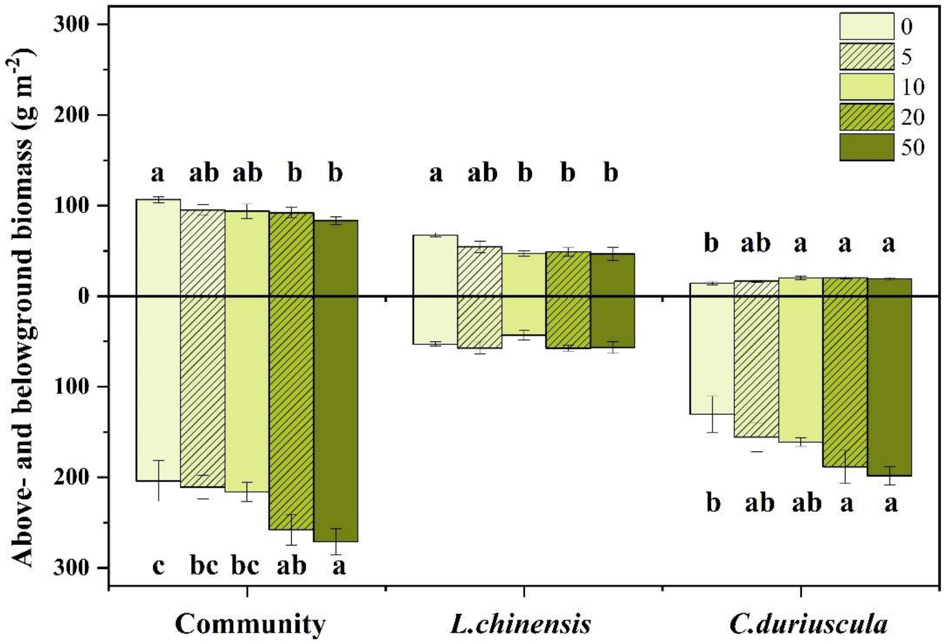

Fig. 1 Effects of S addition on community and species aboveground and belowground biomass. Bars are means ± the standard error. Lower case letters indicate significant difference among treatments ($P < 0.05$).

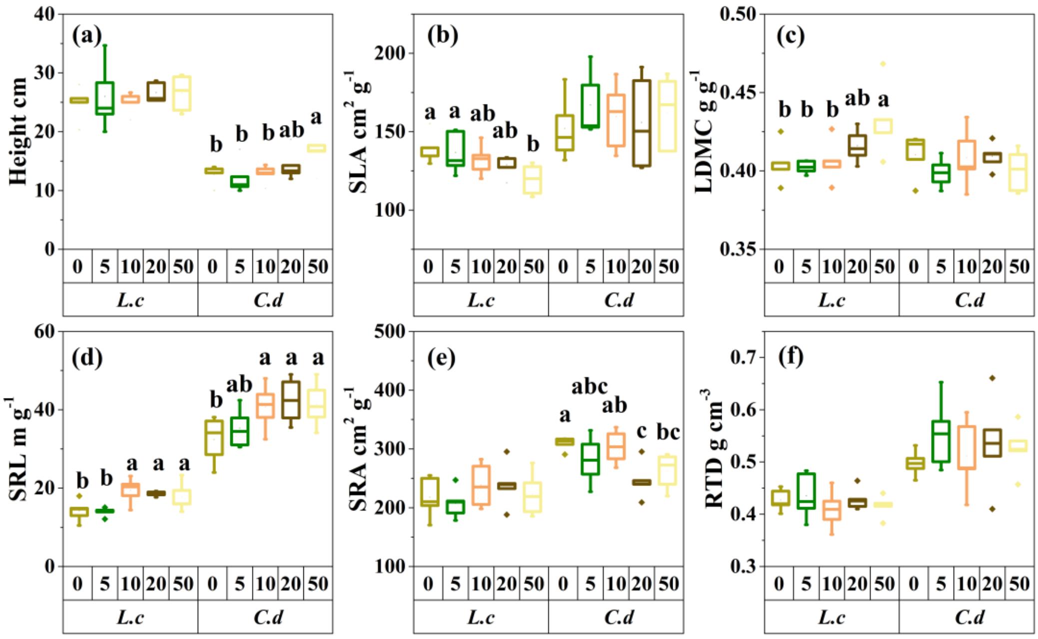

Fig. 2 The response of the morphological traits to S addition for the two dominant species in a meadow steppe. Abbreviations: SLA, Specific leaf area; LDMC, Leaf dry matter content; SRL, specific root length; SRA, specific root area; RTD, root tissue density; *L.c*, *L. chinensis*; *C.d*, *C. duriuscula*. Different letters above the bars indicate significant influence among the S-addition level by one-way ANOVA at $P < 0.05$.

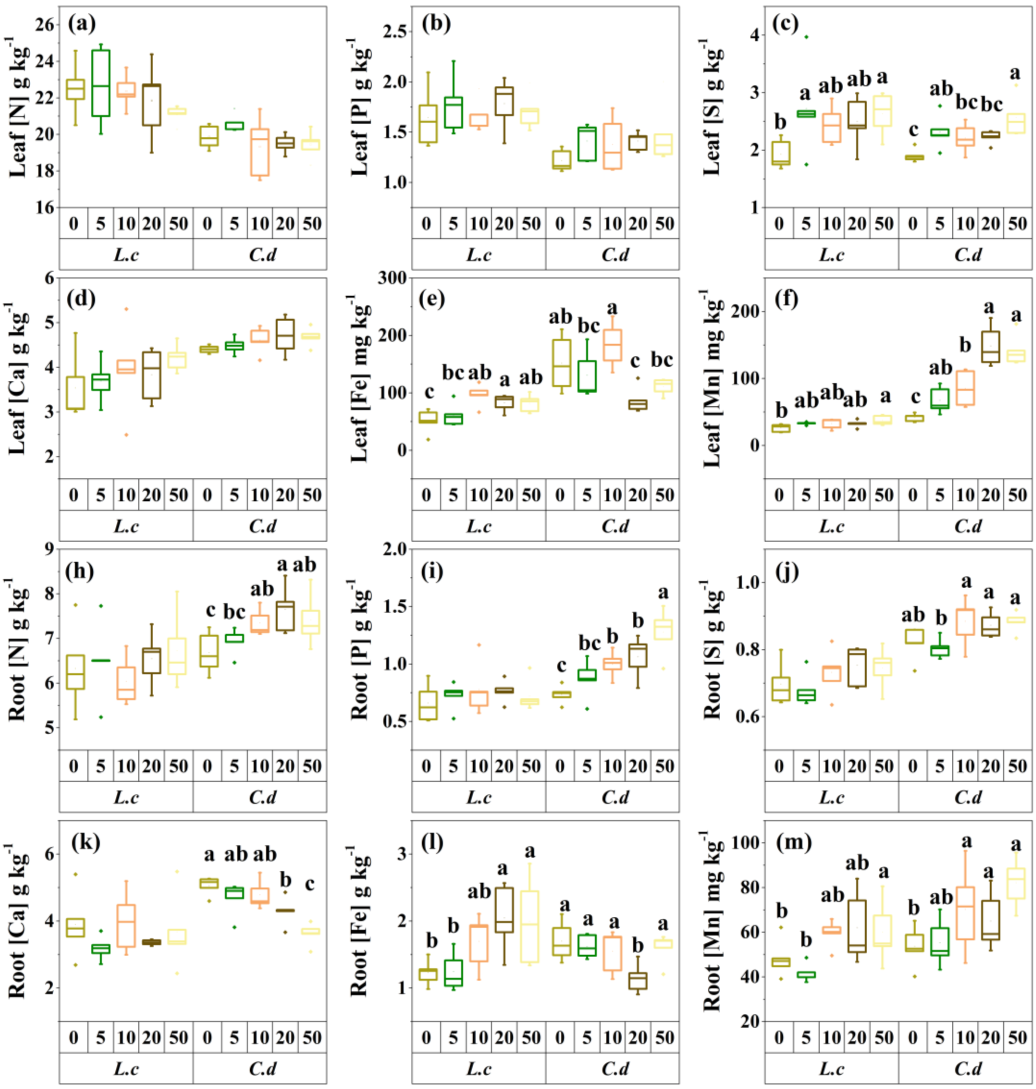

Fig. 3 The response of the chemical traits to S addition for the two dominant species in a meadow steppe. Abbreviations: Leaf [N], leaf N concentration; Leaf [P], leaf P concentration; Leaf [S], leaf S concentration; Leaf [Ca], leaf Ca concentration; Leaf [Fe], leaf Fe concentration; Leaf [Mn], leaf 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 $P < 0.05$.

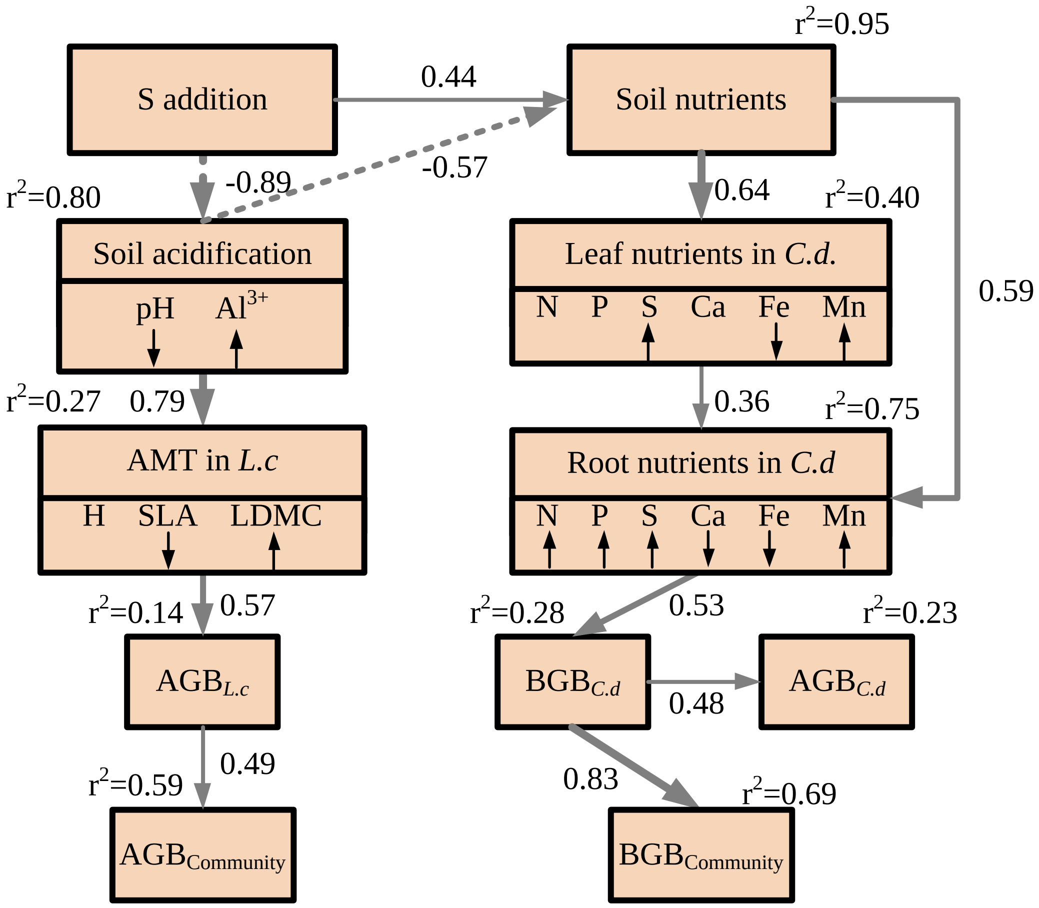

Fig. 4 Structural equation model of S addition on plant community biomass through the plausible pathways. Square boxes indicate the included variables in the analysis: S addition; Soil nutrients include soil NH_4^+-N and NO_3^--N concentrations, soil available phosphorus, soil available sulfur; soil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

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

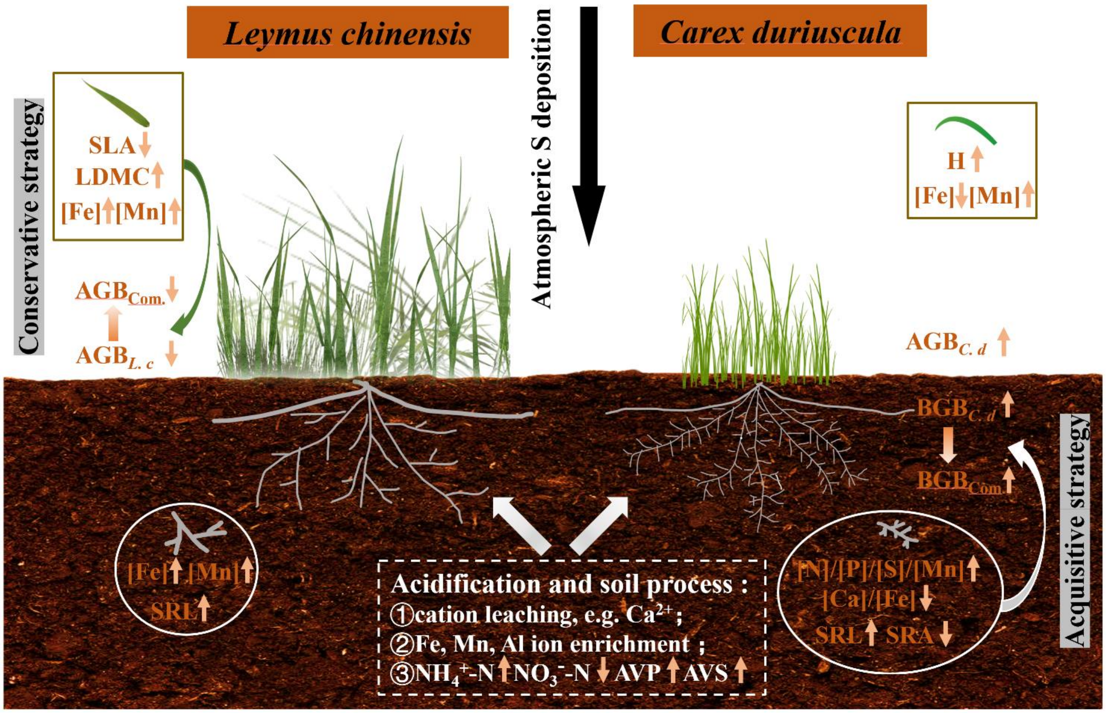

Fig. 5 Schematic diagram illustrating the ecological effects of S-induced soil acidification on above- and belowground biomass and traits of two dominant species in a meadow steppe. ↑ = increase in response to S addition; ↓ = decrease in response to S addition; Com. = Community; AVP = Soil available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

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}.