Dear Professor Nobuhito Ohte,

Thank you very much for sending us the decision on our manuscript. We have revised the manuscript according to reviewer's comments and suggestions. We hope that you will find the new version of our manuscript is now readily suitable for publication in Biogeosciences. In this revised version, changes to our manuscript within the document were all highlighted via track changes. We addressed all queries sequentially as listed below, the original comments are in black, and our responses are in blue.

Queries from the reviewer:

1. P.11 L10: Soluble S or labile S may be better than water-soluble S, because $CaCl_2$ is not just water.

Response: Thanks for the helpful suggestion. We re-defined the $CaCl_2$-extractable fraction as "soluble S" and changed it in the manuscript.

2. P.11 L15-17: Although Ca^{2+} can depress the concentration of organic matter, it could not completely remove organic matter. That's why you used H_2O_2 to remove organic matter, right? And how is HCl extraction? It does not include Ca^{2+}.

Although below is just a suggestion, if you have the next opportunity of the experiment, it is better to apply another method, for example using a resin like DAX-8.

Response: We agree with the reviewer that H_2O_2 digestion could oxidized a part of organic S, especially in HCl extraction without presence of Ca^{2+}. Thus we discussed this shortcoming in the Discussion section "For insoluble S fraction, cautions should be paid to the fact that a small part of organic S might be oxidized into inorganic S during H_2O_2 (1 ml) digestion (Williams and Steinbergs, 1959)." (P.20 L17-20).

We appreciate the reviewer's suggestion for applying another method, for example using a resin like DAX-8, if we have the next opportunity of the experiment.

3. P.22 L10-11: Please add corresponding No. of figure or table. Figure 3?

Response: We added "Figure 3" as suggested. (P.22 L14)

4. P.22 L6: Available S concentration itself is mainly determined by soil characteristics especially amount of adsorption material. Perhaps what you want to say is a change in concentration?

Response: As per suggestion, we replaced "Available S concentration" with "A change of available S concentration" (P.22 L9).

5. P.22 L16-17: How does the result of the control plot support your hypothesis? The explanation is insufficient.

Response: We clarified the description into "The results in the control plots supported this explanation, with lower concentrations of adsorbed S, available S and total inorganic S under low N frequency as compared to high frequency (especially in unmown plots)." (P.22 L20- P.23 L2).

With above corrections, the manuscript is hereby resubmitted to the journal. We are thankful for the reviewers' work and glad to respond any further questions that you have. We look forward a positive response from you.

Thanking you,

Ruzhen Wang

ruzhenwang@iae.ac.cn

Institute of Applied Ecology, Chinese Academy of Sciences

Frequency and intensity of nitrogen addition alter soil inorganic sulfur fractions but the effects vary with mowing management in a temperate steppe

Tianpeng Li [a, 1], Heyong Liu [b, 1], Ruzhen Wang [a,*], Xiao-Tao Lü [a], Junjie Yang [c], Yunhai Zhang [c], Peng He [a], Zhirui Wang [a], Xingguo Han [c], Yong Jiang [a,*]

[a] Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110016, China

[b] College of Land and Environment, Shenyang Agricultural University, Shenyang 110866, China

[c] State Key Laboratory of Vegetation and Environmental Change, Institute of Botany, Chinese Academy of Sciences, Beijing 100093, China

[1] These authors contribute equally to this work.

[*] Correspondence to:

Dr. Ruzhen Wang, Tel: +86 24 83970603; Fax: +86 24 83970300; E-mail address: ruzhenwang@iae.ac.cn

Dr. Yong Jiang, Tel: +86 24 83970902; Fax: +86 24 83970300; E-mail address: jiangyong@iae.ac.cn

Abstract

Sulfur (S) availability plays a vital role in driving functions of terrestrial ecosystems, which can be largely affected by soil inorganic S fractions and pool size. Enhanced nitrogen (N) input can significantly affect soil S availability, but it still remains largely unknown if the N effect varies with frequency of N addition and mowing management in grasslands. To investigate changes in soil S pool and inorganic S fractions (soluble S, adsorbed S, available S, and insoluble S), we conducted a field experiment with different frequencies (twice *vs.* monthly additions per year) and intensities (i.e. 0, 1, 2, 3, 5, 10, 15, 20, and 50 g N m^{-2} $year^{-1}$) of NH_4NO_3 addition and mowing (unmowing *vs.* mowing) over six years in a temperate grassland of northern China. Generally, N addition frequency, N intensity and mowing significantly interacted with each other to affect most of inorganic S fractions. Specifically, significant increase of soluble S was only found at high N frequency with the increasing intensity of N addition. Increasing N addition intensity enhanced adsorbed S and available S concentrations at low N frequency in unmown plots; however, both fractions were significantly increased with N intensity at both N frequencies in mown plots. High frequency of N addition increased the concentrations of adsorbed S and available S as compared to low frequency of N addition only in mown plots. Changes in soil S fractions were mainly related to soil pH, N availability, SOC and plant S uptake. Our results suggested that N input could temporarily replenish soil available S by promoting dissolution of soil insoluble S with decreasing soil pH and mineralization of organic S due to increasing

plant S uptake. However, the significant decrease of organic S and total S concentrations with N addition intensity in mown plots indicated that N addition together with biomass removal would eventually cause soil S depletion in this temperate grassland in the long term. Our results further indicated that using large and infrequent N addition to simulate N deposition can overestimate the main effects of N deposition and mowing management on soil S availability in semi-arid grasslands.

Keywords sulfur availability, nitrogen deposition, nitrogen input frequency, biomass removal, soil acidification, semi-arid grassland

1 Introduction

Sulfur (S) is an essential nutrient for the metabolism of plants and soil microorganisms by constituting amino acids of cysteine and methionine (Blum et al., 2013). It also plays vital roles in increasing plant nitrogen (N) use efficiency, enhancing crop yield and quality (De Bona and Monteiro, 2010), and reducing plant diseases and heavy metal toxicity (Chiang et al., 2006; Feechan et al., 2005). Plant S deficiency is widely distributed in global ecosystems (Kost et al., 2008; Scherer, 2009), with negative effects on stomatal conductance, photosynthetic rate, and consequently on primary productivity (Juszczuk and Ostaszewska, 2011; Wulff-Zottele et al., 2010). As a macronutrient for plant, S occurs in soils in both organic and inorganic forms. Soil organic S includes ester-bonded S, C-bonded S and residual S, with the former two forms constituting the potential S source for plants. Inorganic S, accounting for approximately 5% of total soil S in most temperate soils (Tabatabai, 2005), generally occurs as bioavailable S (including soluble SO_4^{2-} and adsorbed SO_4^{2-}) and insoluble S (SO_4^{2-} coprecipitated with $CaCO_3$ or co-crystallization with cations) (Fig. 1; Tisdale et al., 1993). Soluble S is the most active fraction with high mobility in soil pore water and prone to leaching loss (Fig. 1), while S adsorption helps to retain SO_4^{2-} from hydrologic leaching (Riscassi et al., 2019). Transformations of inorganic S fractions govern soil S availability which are closely associated with plant-soil interactions (Kertesz and Mirleau, 2004; Juszczuk and Ostaszewska, 2011). For instance, the reversible processes of both S adsorption and dissolution (Fig. 1) are pH dependent

(McGill and Cole, 1981; Tabatabai, 2005) and can be accelerated/ decelerated by microbial S immobilization and plant S demand. Therefore, changes in soil inorganic S pool play a major role in S dynamics which can further impact the mineralization of organic S and plant growth (Kertesz and Mirleau, 2004). A deeper understanding on transport and transformation of soil inorganic S fractions is essential for better predicting S supply to plants under global change scenarios.

As mainly caused by increasing atmospheric N deposition and fertilization, enhanced N inputs result in severe ecological problems in temperate ecosystems worldwide (Bobbink et al., 2010), which is predicted to deteriorate in the coming decades, especially in developing countries (Dentener et al., 2006). Higher N input may increase plant S uptake and affect soil S turnover by enhancing primary productivity, especially in N-limited regions (De Bona and Monteiro, 2010; Harpole et al., 2007; Phoenix et al., 2012; Wang et al., 2015). Nitrogen input can promote S supply by stimulating the mineralization of C-bonded S (into the form of SO_4^{2-}-S) (De Bona and Monteiro, 2010) and abiotic dissolution of mineral-bound S under the N-induced soil acidification (Wang et al., 2016). However, results from an 80-year fertilization experiment showed that N addition did not change the concentrations of soil inorganic and organic S (Yang et al., 2007). Nitrogen inputs may have negative effects on S cycling rate due to the inhibitation of arylsulfatase activity (Chen et al., 2016). Therefore, soil S availability is mainly associated with soil pH and mineralization of soil organic matter (SOM) under N enrichment, but it is still poorly

understood for its relationship with inorganic S fractions.

Studies simulating N deposition commonly add N as an intensive and pulsed input (Smith et al., 2009). However, natural N deposition occurs more frequently and evenly in small events (Aneja et al., 2001). Low frequency of N addition increases plant biomass (Barton et al., 2008; Bilbrough and Caldwell, 1997) and ammonia volatilization (Zhang et al., 2014a) but decreases soil pH (Wang et al., 2018) and plant N concentrations (Cheng et al., 2009) as compared to high frequency. Low frequency of N addition has been reported to over-estimate the effects of N deposition on plant species diversity (Zhang et al., 2014b). Therefore, lower soil pH and enhanced plant biomass could possibly promote inorganic S dissolution and plant S retention as affected by low frequency of N addition. Though these studies demonstrated that N input frequencies alter factors associated with transformation of soil S fractions (i.e. soil pH, plant uptake), how N input frequency influences transformation of soil inorganic S fractions still remains largely unknown.

Mowing is a common management practice for hay harvesting in temperate grasslands (Bremer and Ham, 2002; Zhang et al., 2017a), which greatly reduces nutrient return from plant residues (Janzen and Ellert, 1998). Persistent harvesting and mowing could decline incorporation of plant S into soil, break the natural cycling of S, thus cause depletion of soil inorganic S (Solomon et al., 2001). Moreover, mowing can alter soil S mineralization and immobilization by changing soil moisture, microbial activity, and plant biomass allocation (Barrow, 1960). Under N enriched conditions,

mowing would aggravate depletion of soil S pool by removing more plant biomass and plant S out of ecosystems as compared to ambient N condition. The effects of biomass removal on soil inorganic S fractions remain poorly understood, while, to our knowledge, interactive N-mowing effect has not been explored in temperate grassland ecosystems.

Temperate grasslands, which account for 8% of the earth's land surface (White et al., 2000) with important ecological function and economic value, play an important role in global S cycle. Primary productivity of temperate grasslands is mainly limited by N availability and typically sensitive to N input (Niu et al., 2010; Yang et al., 2012). Low background N deposition in temperate grasslands of Inner Mongolia (< 1.5g N m^{-2} yr^{-1}) makes this area an ideal place to investigate ecosystem responses to N enrichment (Zhao et al., 2017). For better understanding soil S supply and turnover under mowing and different intensity and frequency of N addition, a field experiment was conducted to investigate soil inorganic S fractions and their transformations in a temperate steppe of Inner Mongolia. We hypothesized that 1) higher intensity of N addition would increase available S (soluble S and adsorbed S) concentration by promoting insoluble S dissolution with drop of soil pH and enhancement of plant residue return and organic S mineralization; 2) the increase of available S and decrease of insoluble S would be more pronounced with low frequency of N addition due to lower soil pH condition than the high frequency; 3) mowing would decrease soil inorganic S fractions resulting from reduced plant residue return, and such effect

would be exacerbated with increasing N addition intensities due to enhanced plant S retention.

2 Materials and methods

2.1 Site description and experimental design

The study site (43°13′ N, 116°14′ E) is a typical temperate semi-arid steppe at the Inner Mongolia Grassland Ecosystem Research Station (IMGERS) in the Xilin River watershed, Inner Mongolia, China. The mean annual air temperature is 0.9 ℃, varying from -21.4 ℃ in January to 19.7 ℃ in July. The long-term mean annual precipitation is 351.4 mm, about 72.8% of which is concentrated from May to August, according to the data from 1980 to 2013 (monitored and provided by IMGERS). The steppe is dominated by *Leymus chinensis*, *Stipa grandis*, *Agropyron cristatum*, and *Koeleria cristata*. The soil was classified as a Haplic Calcisol by the Food and Agriculture Organization of the United Nations (FAO) soil classification system, with a depth of 100–150 cm and a composition of 21% clay, 60% sand, and 19% silt on average (Hao et al., 2013). The site experienced an uncontrolled heavy sheep grazing since 1980s and has been fenced since 1999. No fertilizer was applied before the experiment was conducted.

The experiment was set up in 2008 following a randomized block design. There were nine N addition intensities (0, 1, 2, 3, 5, 10, 15, 20, 50 g N m^{-2} $year^{-1}$) crossed with two N addition frequencies (2 times $year^{-1}$ *vs*. 12 times $year^{-1}$) and two mowing regimes (unmowing and mowing). Currently, infrequent N addition (i.e. 1 or 2 times

year^{-1}) is commonly used in manipulative experiments to mimic N deposition, which is quite different from natural N deposition occurring continuously throughout the year (Smith et al., 2009). Therefore, a more frequent and even way of N addition (i.e. 12 times year^{-1}) was set to simulate natural N deposition (Aneja et al., 2001) and to compare whether changing frequency of N input affects the grassland ecosystem. Higher rates of N addition were used to mimic accumulative N deposition in the long-term and/or extreme N inputs in the future of the grassland (Zhang et al., 2017b). NH_4NO_3 (> 99.5%) was added in wet and dry forms to simulate the wet and dry N deposition, respectively. For the high-frequency treatments, NH_4NO_3 was added monthly since 1st September 2008. For the low-frequency treatments, NH_4NO_3 was added on the 1st of June and November since November 2008. During the growing season (from May to October), N was added in wet form by mixing NH_4NO_3 with purified water (9.0 L water in total for each plot, either 9.0 L once in June for low frequency or 1.5 L monthly from May to October for high frequency) and then sprayed evenly with a sprayer. To simulate dry N deposition, NH_4NO_3 was mixed with treated sand to make sure even-fertilizing in non-growing season from November to next April. Specifically, 500 g sand was used once in November for low-frequency treatments, and 80 g sand was used monthly from November to next April for high-frequency treatments and then broadcast evenly in every plot. The sand used in this experiment was sieved through a 1-mm sieve, dipped in hydrochloric acid, washed in purified water and then oven-dried at 120 ℃ for 48 h to remove

acid-soluble S. Annual mowing was conducted at the end of August using a hay mower at 10-cm height to simulate the local hay-cutting management. The aboveground plant residue was taken away immediately after mowing. We also set an unnamed control without any treatment (N addition, mowing, water or sand addition) to determine the impacts of water and sand addition, which was also compared with mowing treatment without any other treatments. Thus, there were 38 treatments with 10 replicate blocks for every treatment. Each plot is 8 m × 8 m and separated by a 1-m buffer zone.

2.2 Plant and Soil sampling

We assessed aboveground biomass in each plot by clipping a 1 m × 1 m quadrat above soil surface in late August 2014. All the living plants were clipped and sorted by species, dried and weighed. The plant samples were washed using deionized water and then dried to constant weight at 65 ℃ for 48 h.

Soil samples were collected from each of the 380 plots at the beginning of August 2014 (i.e. after six years of treatments). A mixed sample was taken randomly from five cores of the topsoil (0-10cm) within each plot. Then, samples were passed through a 2-mm sieve immediately to remove the plant residues and then air-dried for further analysis.

2.3 Measurement of soil chemical properties

Soil pH was determined in a soil slurry at 2.5:1 (w/v) water: soil ratio by a digital pH meter (Precision and Scientific Crop., Shanghai, China) and the data are given in

Table 1 reported by Wang et al. (2018). The concentration of soil organic carbon (SOC) in the topsoil was determined by oxidation using a mixture of $K_2Cr_2O_7$ solution and sulphuric acid and titration using $FeSO_4$ (Nelson and Sommers, 1982). Soil total inorganic nitrogen (TIN) concentration was calculated as the sum of ammonium and nitrate, which was extracted with 2 *M* KCl and determined using a continuous flowing analyzer (SANplus segmented flow analyzer, Scalar, The Netherlands). Soil pH, SOC and TIN were previously reported in Wang et al. (2018). Soil total sulfur (TS) concentration was analyzed with an elemental analyzer (Vario MACRO cube, Elementar Analysensysteme GmbH, Germany).

A schematic diagram was shown to illustrate the transformation processes and extraction/ calculation procedures of the S fractions (Fig. 1). Soluble S, available S and total inorganic S were extracted with 0.01 *M* $CaCl_2$, 0.01 *M* $Ca(H_2PO_4)_2$ and 1 *M* HCl at a 5:1 (w/v) water: soil ratio, respectively (Roberts and Bettany, 1985). Briefly, 5.0 g of the air-dried soil was mixed with 25 ml extractant and shaken at 400 rpm for 60 min at 25 ℃. The extracts were filtered and digested with 1 ml of 30% H_2O_2 for 20 min to remove the color of soil organic matter. The Ca^{2+} in extractants of $CaCl_2$ and $Ca(H_2PO_4)_2$ can effectively depress the solubility of organic matter during the extraction. The cooled solutions were then mixed with 0.5 ml of HCl (1:4) and 1 ml of gum acacia solution (to stabilize the suspension) successively and adjusted to 25 ml. The SO_4^{2-} in all the extracts was quantified by turbidimetry with 0.5 g of $BaCl_2$ crystals at 440 nm using a UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan)

(Tabatabai and Bremner, 1972). Adsorbed S, total inorganic S and organic S concentrations were calculated as follows:

$$\text{adsorbed S} = \text{available S} - \text{soluble S} \quad (1)$$

$$\text{insoluble S} = \text{total inorganic S} - \text{available S} \quad (2)$$

$$\text{organic S} = \text{total S} - \text{total inorganic S} \quad (3)$$

We measured total S concentration in two dominant species of *Leymus chinensis* and *Stipa grandis* in N addition plots of 0 and 15 g N m^{-2} $year^{-1}$ under low and high frequency of N addition with and without mowing. Briefly, 0.3 g plant samples were acid digested with a 1:2 (v/v) mixture of 65% nitric acid and 72% perchloric acid around 235 ℃ (Soon et al., 1996). The S concentration of digestion solution was quantified by turbidimetry at 440 nm using a UV-VIS spectrophotometer (UV-1700, Shimadzu, Tokyo, Japan). Plant S uptake of dominant species was calculated as follow:

$$S_{uptake} = \sum(S_i \times m_i) \ , \quad (4)$$

where S_i represents S concentration in plant species i and m_i represents aboveground biomass of the corresponding species.

2.4 Statistical analysis

The Kolmogorov-Smirnov test and Levene's test were executed to determine the normality of data and homogeneity of variances, respectively. The TIN was log_{10}-transformed for homogeneity. We used three-way ANOVAs to determine the effects of N addition intensity, N addition frequency, mowing, and their interactions

on the concentrations of soil inorganic S fractions and total S. Student's t-test was performed to estimate the difference in plant biomass and S uptake between two N frequencies within each N addition intensity with or without mowing. Correlation analyses were conducted to estimate the relationships between soil parameters and concentration of soil S fractions. The proportion of S fractions relative to total S was calculated and Duncan's multiple range test was employed to estimate differences among treatments. All the analyses above were conducted using SPSS 18.0 (SPSS Inc., Chicago, IL, USA) and all statistical significance was accepted at $P < 0.05$. Moreover, we calculated the response ratio of available S concentration to mowing practice as follow:

$$\text{Response ratio} = \frac{S_{mown}}{S_{unmown}} \tag{5}$$

where S_{mown} and S_{unmown} represent available S concentration in mown and unmown plots, respectively. Weighted log response ratio (log_eRR) and its 95% confidence intervals for the effect of mowing were calculated using the metafor package in R software, ver. 3.5.1 (Viechtbauer, 2010). Confidence intervals not overlapping zero indicated significant mowing effects on available S concentration.

Structural equation modeling (SEM) was conducted to examine the direct and indirect strength of N addition intensities and frequencies on soil inorganic S fractions through the changes in soil parameters with the AMOS 24.0 (Amos Development Co., Greene, Maine, USA). Data from all the treatment were pooled for the SEM analysis. A *priori* model (Fig. S1a) was constructed by assuming that soil S fractions could be

directly affected by N addition frequency, intensity and mowing, or indirectly by altering soil pH, plant biomass return and organic S mineralization (see hypotheses in the Introduction). Insignificant pathways and parameters that had no effect on inorganic fractions were excluded from the model sequentially to obtain the final model (Fig. S1b, c). Data were fitted to the model using the maximum likelihood estimation method. We used χ^2 -test ($P > 0.05$), root square mean errors of approximation (RMSEA, < 0.08), and Akaike Information Criteria (AIC) to evaluate the adequacy of the model.

3 Results

3.1 Effects of N addition and mowing on soil characteristics

Soil pH significantly decreased and TIN increased along the N gradient under all treatments of addition frequency and mowing regime (Table 1). Soil pH exhibited a sharper decrease in low N frequency as compared to high N frequency in unmown plots, especially at N addition intensities of 10 and 15 g N m^{-2} $year^{-1}$. Low frequency of N addition increased TIN in both unmown and mown plots at high N levels ($P < 0.05$ at 50g N m^{-2} $year^{-1}$). SOC decreased along the N gradient only under low N frequency in mown plots.

3.2 Effects of N addition and mowing on soil inorganic S fractions

Soil soluble S

Mowing significantly decreased soil soluble S concentration by up to 47% across N frequency and intensity (Fig. 2a, b; Table 2). High frequency of N addition

significantly increased soluble S concentration by up to 90% with both unmown and mown plots, resulting in a significant M×F effect (Fig. 2a, b; Table 2). At high frequency of N addition, N addition intensity increased soluble S for both unmown (Fig. 2a, $P = 0.004$) and mown plots (Fig. 2b, $P = 0.001$), causing a significant F×N effect (Table 2).

Soil adsorbed S

Soil adsorbed S concentration was significantly affected by mowing treatments and intensity of N addition (Table 2). Mowing significantly decreased soil adsorbed S concentration at low N addition frequency (Fig. 2c, d; Table 2). There was no effect of N addition frequency on adsorbed S (Table 2). As compared to control plot, N addition intensity increased soil adsorbed S concentration at low frequency of N addition in unmown plots (Fig. 2c, $P < 0.01$) and at both low and high frequency of N addition (Fig. 2d, P=0.04 and 0.01, respectively) in mown plots, causing significant M×F and F×N effects (Table 2).

Soil insoluble S

Mowing decreased soil insoluble S concentration by up to 91% across N treatments (Fig.2 e, f). There was no significant effect of N addition frequency on soil insoluble S (Table 2). Intensity of N addition decreased soil insoluble S concentration at both low and high N frequency for unmown plots (Fig.2e; Table 2, $P = 0.01$ and <0.01, respectively), but only at high N addition frequency for mown plots (Fig.2f, $P < 0.01$), resulting in significant M×F and M×F×N effects on soil insoluble S concentration

(Table 2).

3.3 Effects of N addition and mowing on soil available S

Mowing significantly decreased soil available S concentration by up to 43% and 40% across N intensity at low and high N addition frequency, respectively (Fig. 3a, b; Table 2). For mown plots, high frequency of N addition significantly increased soil available S concentration by up to 57% comparing to low N frequency across N addition intensity (Fig. 3b; Table 2). High intensity of N addition increased soil available S concentration at lower frequency of N addition for unmown plots from 15.9 to 24.0 mg kg soil^{-1} (Fig. 3a, $P < 0.01$), and at both low (from 10.7 to 15.6 mg kg soil^{-1}, $P = 0.01$) and high (from 12.0 to 23.0 mg kg soil^{-1}, $P < 0.01$) frequencies of N addition for mown plots (Fig. 3b). This resulted in significant interactive effects of M×F, F×N, and M×F×N on soil available S (Table 2).

Nitrogen addition intensity enhanced the negative effect of mowing on soil available S concentration at low N frequency (Fig. 3c, $P < 0.01$). However, negative mowing effect on available S concentration decreased along the increasing N addition intensity and even turned into positive at 15, 20 and 50 g N m^{-2} year^{-1} at high frequency of N addition (Fig. 3c, $P < 0.01$). Proportion of available S relative to total S increased with increasing N intensity at low N frequency for unmown plots (Fig. S2a) and at both low and high N frequencies for mown plots (Fig. S2c,d).

3.4 Effects of N addition and mowing on soil total inorganic S, total S, plant biomass and plant S uptake

Mowing significantly decreased total inorganic S at both low and high frequency of N addition by up to 38% (Fig. 4a, b). High frequency of N addition significantly increased soil total inorganic S concentration by as much as 15% (unmown plots) and 26% (mown plots) comparing to low N frequency (Fig. 4a, b). Soil total inorganic S concentration decreased with increasing N intensity (except for 20 and 50 g N m^{-2} $year^{-1}$) for both N frequencies in unmown plots, while it showed no response in mown plots. Soil organic and total S concentrations were not affected by intensity of N addition, frequency of N addition and mowing treatment as suggested by three-way ANOVAs (Table 2). However, the mean values of soil organic and total S concentration tended to decrease with increasing N addition intensity as indicated by its linear correlation with N addition intensity at both low and high N addition frequency in mown plots (Fig. 4d, f). Mowing significantly decreased the proportion of total inorganic S relative to total S and increased organic S proportion (Fig. S2a *vs.* 2c and S2b *vs.* 2d).

High intensity of N addition increased aboveground biomass regardless of N addition frequency and mowing management (Fig. S3). Low frequency of N addition increased aboveground biomass at 2 and 15 g N m^{-2} $year^{-1}$ as compared to high frequency of N addition in unmown plots. Sulfur uptake of *Leymus chinensis* and *Stipa grandis* showed no response to mowing management in plots without N addition, but it increased with mowing under both frequencies of N addition. Low frequency of N addition increased S uptake of dominant species at 15 g N m^{-2} $year^{-1}$ in both mown

and unmown plots (Fig. 5). Nitrogen addition intensity increased plant S concentration of two dominant species (Fig. S4) and relative biomass proportion of *Stipa grandis* (Fig. S5).

3.5 Relationships between soil characteristics and S fractions

Soil pH was negatively correlated with adsorbed S and available S concentrations under both low (Fig. 6a) and high frequency of N addition (Fig. 6b) across intensity of N addition and mowing treatments (all $P < 0.01$). However, insoluble S was positively correlated with pH under both N frequencies. Soil TIN was positively correlated with adsorbed S and available S but negatively correlated with insoluble S concentration under both low and high frequency of N addition. Soil organic carbon was positively correlated with soluble S under both N frequencies. Insoluble S was negatively correlated with adsorbed and available S under both N frequencies (all $P < 0.01$, Fig. 6a, b). Organic S concentration was negatively correlated adsorbed S and available S at high frequency of N addition across N intensity and mowing treatments ($P < 0.01$, Fig. 6b).

Results of SEM showed that N addition intensity affected soil inorganic S fractions through altering soil pH, plant biomass, and SOC (Fig. 7a, b). Plant biomass, representing plant S uptake showed a negative effect on adsorbed S concentration. With increasing intensity of N addition, higher plant biomass increased soil organic carbon which elevated soil soluble S concentration (Fig. 7b). Frequency of N addition directly and positively affected soil soluble S (standardized total effect size: 0.26) but

indirectly influenced adsorbed S (effect size: 0.07) and inorganic S (effect size: 0.05) concentrations by changing soil pH (Fig. 7c). Consistent with the results of simple regressions, soil pH had negative effect on soil adsorbed S which was positive for soil insoluble S. Mowing decreased soluble S, adsorbed S, and insoluble S concentrations (Fig. 7d).

4 Discussion

4.1 Positive effects of N addition intensity on soil available S resulted from higher abiotic dissolution, adsorption and potential organic S mineralization

Consistent with the first hypothesis, increasing N addition intensity enhanced soil available S fractions, especially adsorbed S concentrations. In this study, higher adsorbed S under N addition was mainly derived from higher ability of sulfate adsorption with decrease of soil pH (Fig. 7b), which was in line with Nodvin et al. (1986). Adsorption of SO_4^{2-} is pH dependent as anionic groups from SOM compete with SO_4^{2-} for adsorption sites on Fe- and Al-hydroxides (Johnson and Todd, 1983). Under acidic conditions, soil matrix can provide adsorption sites with positive charges to attract the negatively charged SO_4^{2-} (Tabatabai, 2005). Therefore, lower soil pH contributed to higher adsorbed S concentration via enhancing adsorption strength and increasing electrostatic potential of the adsorption sites under higher intensity of N addition (Scherer et al., 2012). However, unaffected adsorbed S in unmown plots could be possibly due to the fact that soil pH tended to be higher at high N frequency as compared to low N frequency at the same N addition level (significant at 10, 15

and 20 g N m^{-2} $year^{-1}$, see Table 1). Moreover, soil pH decreased at a much lower rate along with increasing N addition intensity (significant decrease only detected at 20 and 50 g N m^{-2} $year^{-1}$) under high N frequency comparing to low N frequency. This resulted in weaker S adsorption strength, less SO_4^{2-} release from insoluble S dissolution, and consequently unchanged adsorbed S concentration with increasing N intensity at high N frequency.

Increasing N addition intensity enhanced soil available S fractions partially due to the dissolution of soil insoluble S as suggested by negative correlation between the two S fractions in our study (Fig. 6). Without N deposition, free sulfate in soil could precipitate as calcium-, magnesium- or sodium sulfate and co-crystallize/co-precipitate with $CaCO_3$ (Tisdale et al., 1993), especially in this calcareous soil rich in exchangeable Ca^{2+}, Mg^{2+} and Na^{+} (Wang et al., 2018). Soil acidification induced by N addition can reverse the precipitation process by dissolving $CaCO_3$-sulfate complexes (Chen et al., 1997; Zhang et al., 2016). Therefore, insoluble-S dissolution sequentially enhanced SO_4^{2-} mobility as affected by soil acidification under excessive N input. This postulation was further confirmed by the positive relationships of soil pH and insoluble S at both N frequencies (Fig. 6a, b). For insoluble S fraction, cautions should be paid to the fact that a small part of organic S might be oxidized into inorganic S during H_2O_2 (1 ml) digestion (Williams and Steinbergs, 1959). At low N frequency of N addition, non-significant effect of N intensity on soluble S concentration could probably attribute to the balance between S

adsorption and dissolution processes under soil acidification thus keeping the concentration of soluble S stable. Soluble S is the most active and mobile S fraction in topsoil for it can be easily utilized by plants and leached along with soil pore water (Tabatabai, 2005). The increase of soluble S with high frequency of N addition indicates that N deposition might increase the risk of S losses via leaching and/or surface runoff.

Our results demonstrate that N input increased soil available S potentially by increasing organic S mineralization in mown plots as indicated by the decreased organic S concentration (Fig. 4d). This was consistent with previous observations that N addition enhanced mineralization of organic S to increase S availability by elevating microbial activity (Ghani et al., 1992). Soil N availability has considerable impacts on the mineralization of organic S (Niknahad-Gharmakher et al., 2009). The increase of soil TIN following N input possibly accelerated organic S mineralization in this study. Changes in SOC concentration also contributed to the increase of soluble S (Fig. 7a) as biological mineralization of C-bonded S was positively related to SOC concentration (Niknahad-Gharmakher et al., 2009). Moreover, decrease of soil pH and higher plant S uptake under N input (Fig. 5) could promote biochemical mineralization of organic S via enhancing secretion of arylsulfatase by soil microorganisms (McGill and Cole, 1981). This was further confirmed with Niknahad-Gharmakher et al. (2009) reporting the upregulation of soil organic S mineralization by decrease of soil pH. Elevation of soil S availability via the two

pathways of insoluble S dissolution and organic S mineralization implies that N deposition would substantially promote transportation of soil S into plant biomass in the semi-arid grassland. This finding is relevant to understand the fates of S under ecosystem N enrichment with the presence of other disturbances such as mowing and livestock grazing of grasslands.

4.2 Effects of N addition frequency on soil inorganic S fractions

Partially contrary to our second hypothesis, low frequency of N addition decreased soluble S, adsorbed S, available S and total inorganic S concentrations as compared to high frequency. ~~Soil~~ A change in soil available S concentration was mainly determined by the input from dissolution of insoluble S and output to plant uptake and leaching. A sharper decrease of soil pH with low N frequency relative to high N frequency would result in higher soil available S concentration via enhancing insoluble S dissolution. In contrary, lower available S concentration was found under low N frequency as compared to high N frequency (Fig.3), which could be possibly driven by higher plant S uptake (Fig. 5) surpassing the amount of S dissolution. Leaching loss of SO_4^{2-} was evident with infrequent and extreme rainfall pulses in sandy soils (Eriksen and Askegaard, 2000). Therefore, another potential explanation could be that large-pulse water input at low N frequency resulted in higher leaching loss of available S than the high N frequency of adding small-amount water each time. The results in the control plots supported this explanation, with lower concentrations of adsorbed S, available S and total inorganic S under low N frequency as compared

to high frequency (especially in unmown plots)~~ in the control plots supported this explanation~~. With the increases of N intensity, leaching loss of available S was exacerbated due to enhanced insoluble S dissolution (Fig. 2e, f). Comparing to high N frequency, organic S mineralization did not contribute to lower total inorganic S and available S concentrations at low N frequency for the same N intensity as no difference was detected for organic S concentration between two N frequencies. These results suggest that using low frequency of N addition to mimic N deposition may overestimate insoluble S dissolution (especially in unmown plots) and plant S uptake in temperate grasslands. However, some of the S fractions responded differently to both N intensity and frequency with or without mowing treatment, suggesting that the effects of N addition strongly depended on mowing practice.

4.3 Mowing effect and its interaction with N addition

Mowing decreased inorganic S fractions in all treatments, which supported our third hypothesis. Mowing could alter plant community composition (Lü et al., 2012) and ecosystem nutrient cycling (Koncz et al., 2015). Decreased soil nutrient availability was found under mowing practice in a similar grassland ecosystem resulting from reduced plant residue return (Lü et al., 2012). Similarly, mowing resulted in significant decreases of inorganic S fractions and the proportion of total inorganic S relative to soil total S (Fig. 4a, b; Fig. S2). In contrast, relative organic S proportion increased with mowing treatment (Fig. S2), which indicates that mowing management removes soil S out of the ecosystem having a larger impact on inorganic S

transformation rather than the organic S mineralization (Fig.4a, b *vs.* Fig. 4c, d). This is in contrary with findings of Li et al. (2001), which suggested that over 70% of S removal by harvests was derived from organic S mineralization in an agroecosystem. The difference could probably attribute to relatively lower intensity of plant S uptake and fertility of soil of the grassland ecosystem than the cropland.

Negative mowing effect on soil available S was suggested to be exacerbated due to enhanced plant S uptake coincident with higher plant biomass (Jackson, 2000). We found that combined mowing and N addition treatments tended to increase S uptake of dominant species (Fig. 5). This was probably due to higher S concentration in *Stipa grandis* (Fig. S4) and increases of its biomass proportion after mowing (Fig. S5). However, the exacerbation of negative mowing effect on available S was only detected at low frequency of N addition in this study, and high N frequency alleviated the negative mowing effect and even turned it into positive (> 15 g N m^{-2} yr^{-1}) with the increasing N addition intensity (Fig. 3c). The discrepancy might be recognized as the tradeoff between available S output process and input process and differential pH responses under two N addition frequencies. Therefore, it was reasonable to detect the decrease of soil total S but increase of soluble S and adsorbed S concentrations under N addition with mowing treatment. Our results suggest that the increase of S removal from the grassland ecosystem could temporally stimulate available S formation via abiotic dissolution of insoluble S and mineralization of organic S. In the long term, however, N enrichment together with continuous mowing would cause depletion of S

pool.

5 Conclusions

Increasing the intensity of N input enhanced soil available S fractions through affecting soil pH, plant S uptake, SOC and insoluble S concentration. Dissolution of insoluble S and mineralization of organic S contributed to the increases of soil S availability. Mowing significantly decreased soil inorganic S fractions by reducing S replenishment via plant residue return and such effect was exacerbated with increasing intensity of N addition by enhancing plant S uptake. Frequency of N addition also interacted with mowing to decrease soil adsorbed and available S with higher response ratio under low frequency of N addition. Our results indicated that simulating N deposition using large and infrequent pulses of N could overestimate changes in adsorbed S and available S under unmowing treatment, but underestimate responses of soluble S, adsorbed S and available S concentrations under mowing treatment. Mowing could regulate the effects of N addition intensity and frequency on soil S dynamics in semi-arid grassland ecosystems. Our results provide insights for sustainable grassland management from a perspective of S cycling.

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Table 1 Effects of N addition and mowing practice on soil pH, soil organic carbon (SOC) and total inorganic nitrogen (TIN). Data shown are the mean values, and the values in parentheses are standard errors (n = 10), which were previously reported in Wang et al. (2018).

N intensity		Unmown		Mown	
	(g N $m^{-2}year^{-1}$)	F2	F12	F2	F12
pH	0	7.49 (0.13)a	7.34 (0.08)a	7.38 (0.12)ab	7.47 (0.12)ab
	1	7.60 (0.18)a	7.34 (0.08)a	7.58 (0.13)a	7.28 (0.11)ab
	2	7.40 (0.17)a	7.51 (0.19)a	7.61 (0.18)a	7.64 (0.12)a
	3	7.48 (0.17)a	7.33 (0.14)a	7.36 (0.21)ab	7.34 (0.14)ab
	5	6.78 (0.17)b	6.99 (0.18)a	6.97 (0.18)abc	7.30 (0.14)ab
	10	6.47 (0.19)Bb	7.08 (0.18)Aa	6.80(0.16) bcd	7.01 (0.17) b
	15	6.24 (0.24)Bb	7.08 (0.18)Aa	6.42(0.30)cd	6.48 (0.18)c
	20	5.56 (0.16)Bc	6.22 (0.23)Ab	6.13 (0.31)de	6.08 (0.24)c
	50	5.21 (0.26)c	5.13 (0.15)c	5.54(0.32)e	5.41(0.29)d
TIN (mg kg^{-1})	0	9.30 (0.61)c	10.13 (0.72)d	9.78 (0.65)d	9.20 (0.89)c
	1	10.29 (1.02)c	10.24 (1.00)d	10.13 (0.53)d	9.74 (0.68)c
	2	11.09 (1.00)c	9.64 (0.62)d	10.97 (0.63)d	10.88 (0.82)c
	3	10.19 (1.13)c	10.61 (0.87)d	11.94 (0.80)d	10.75 (0.55)c
	5	14.62 (1.34) c	13.19(0.88) d	14.70(1.22)Ad	10.29(1.00)Bc
	10	17.31 (3.38)c	22.51 (2.47)c	18.54 (2.92)cd	17.80 (2.31)bc
	15	30.69 (5.46)b	31.04 (3.77)b	32.22 (4.41)bc	25.84 (4.44)b
	20	41.03 (5.77)b	36.48 (4.29)b	36.46 (7.05)b	28.05 (3.58)b
	50	104.09(10.62)Aa	74.60 (6.22)Ba	111.94(15.14)Aa	71.93(10.32)Ba
SOC (%)	0	2.42 (0.06)Aa	2.17 (0.05)Bb	2.60 (0.09)Aa	2.22(0.11)Bab
	1	2.45 (0.08) Aa	2.24 (0.06) Bb	2.45 (0.09)ab	2.33 (0.15)ab
	2	2.17 (0.07)b	2.22 (0.06)b	2.36 (0.11)Aabc	2.07 (0.08)Bb
	3	2.16 (0.07)b	2.36 (0.13)ab	2.36 (0.11)abc	2.27 (0.10)ab
	5	2.19 (0.06)Bb	2.53 (0.09)Aa	2.27 (0.08)bc	2.35(0.08)ab
	10	2.26 (0.09)ab	2.39 (0.10)ab	2.25 (0.08)bc	2.47 (0.09)a
	15	2.12 (0.08)b	2.21 (0.11)b	2.14 (0.11)bc	2.35 (0.10)ab
	20	2.43 (0.09)a	2.33(0.08)ab	2.10 (0.09)c	2.27(0.11)ab
	50	2.31 (0.05)ab	2.18 (0.10)b	2.28 (0.07)bc	2.36 (0.07)ab

Notes: Different letters indicate significant differences among means of different N addition frequencies with and without mowing practice separately under the same N intensity (uppercase letters) and among means of different N addition intensities within one frequency of N addition with or without mowing (lowercase letters). F2 and F12 represent low and high frequency of N addition, respectively.

Table 2 Results of three-way ANOVAs (*F* value) for the effects of mowing practice (M) and N addition frequency (F) and intensity (N) on soil sulfur (S) fractions and total S concentration.

	SS	AdS	AvS	InS	TIS	OS	TS
M	74.46**	60.40**	145.25**	28.32**	449.97**	3.43	2.91
F	120.82**	0.23	31.59**	0.35	57.77**	0.17	0.08
N	3.95**	11.36**	16.64**	21.83**	5.94**	1.45	1.48
M×F	4.80*	5.98*	12.59**	9.62**	0.35	0	0
M×N	1.22	0.84	0.66	1.67	3.91**	0.39	0.80
F×N	5.05**	4.83**	2.53*	2.55**	0.47	0.81	0.39
M×F×N	1.45	6.65**	6.34**	8.37**	1.05	1.38	1.42

Note: SS represents soluble S, AdS for adsorbed S; AvS for available S, InS for insoluble S, TIS for soil total inorganic S, OS for organic S, and TS for soil total S.

* and ** represent significance level at 0.05 and 0.01, respectively

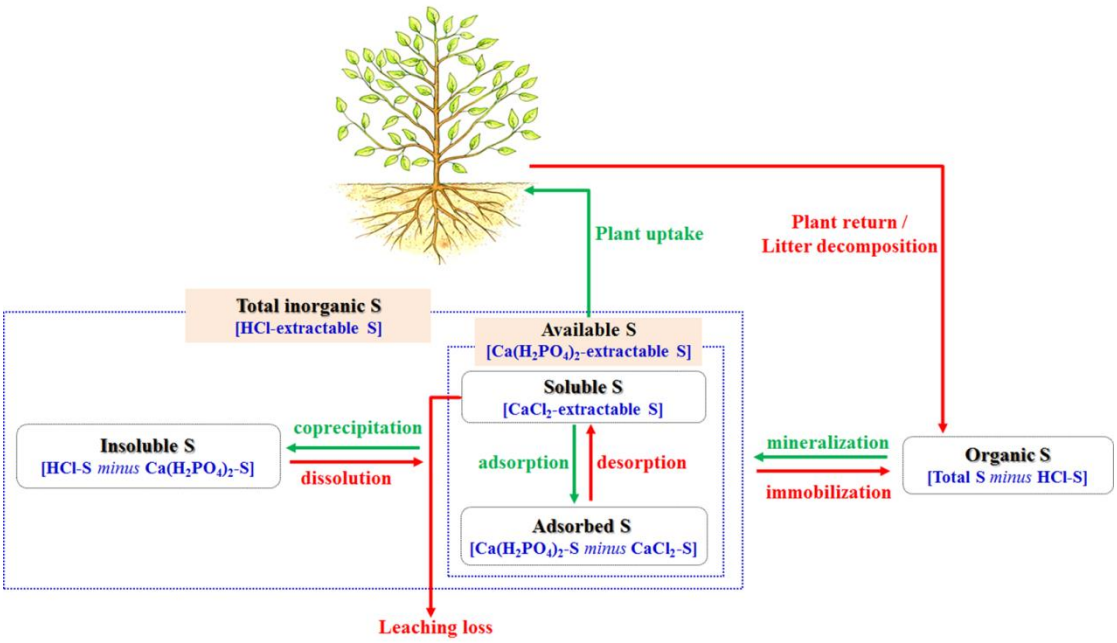

Figure 1 Conceptual scheme depicting the transformation of sulfur (S) fractions in aerobic calcareous soils. Arrows indicate processes affected the concentration of one S fraction with green and red colors representing opposite transformation processes. Below each S fraction, characters with blue color represent that the S fraction was directly measured from extraction or indirectly calculated from analyzed fractions. Specifically, soluble S ($CaCl_2$-S), available S ($Ca(H_2PO_4)_2$-S) and total inorganic S (HCl-S) was directly extracted by $CaCl_2$, $Ca(H_2PO_4)_2$ and HCl, respectively. Adsorbed S was calculated by $Ca(H_2PO_4)_2$-S minus $CaCl_2$-S, insoluble S as HCl-S minus $Ca(H_2PO_4)_2$-S, and organic S as total S minus HCl-S.

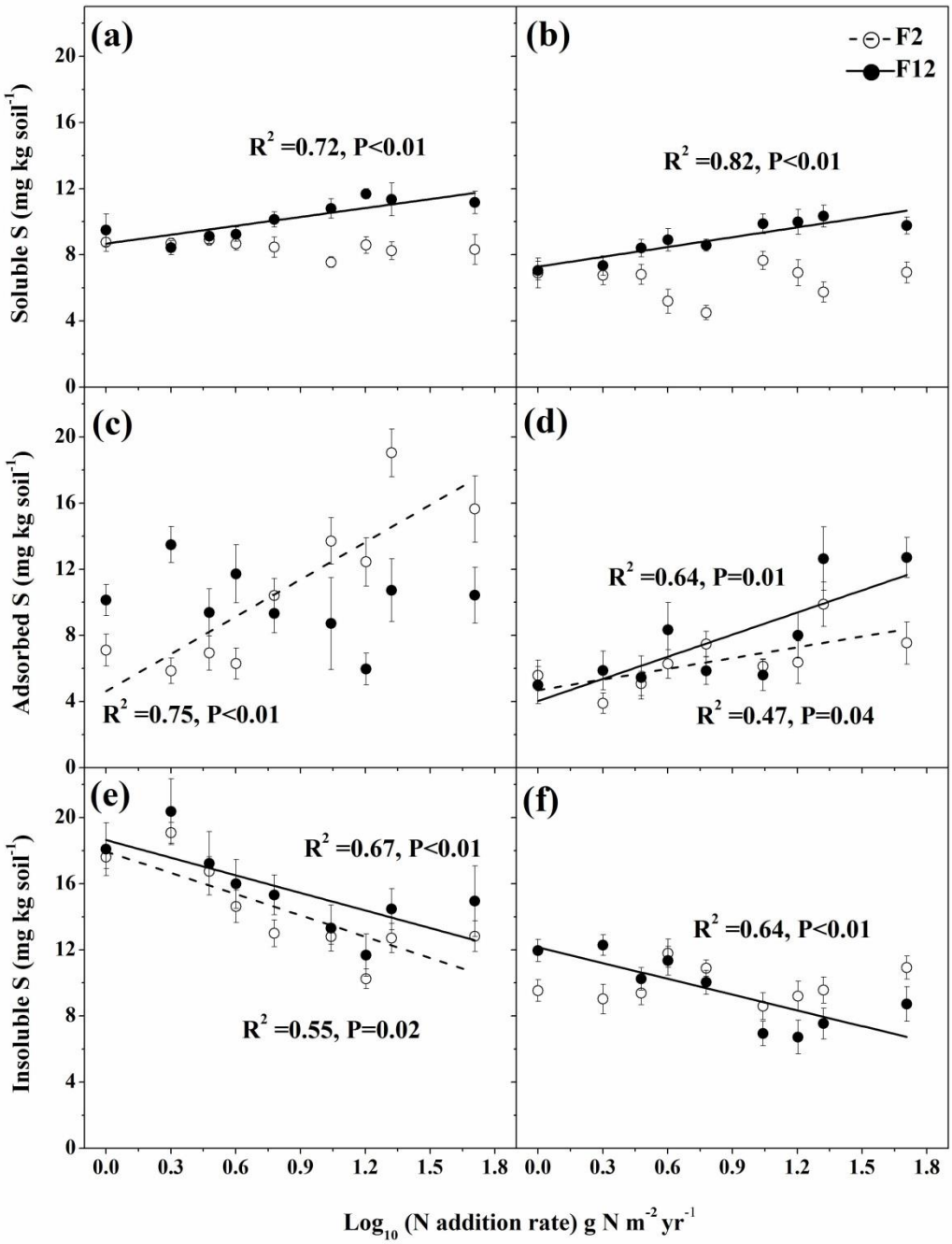

Figure 2 Effects of N addition intensity and frequency on concentrations of soil soluble S (a, b), adsorbed S (c, d) and insoluble S (e, f) in unmown (left figures) and mown plots (right figures). Dashed and solid regression lines represent 2 and 12 N additions year^{-1}, respectively. Error bars indicate standard error.

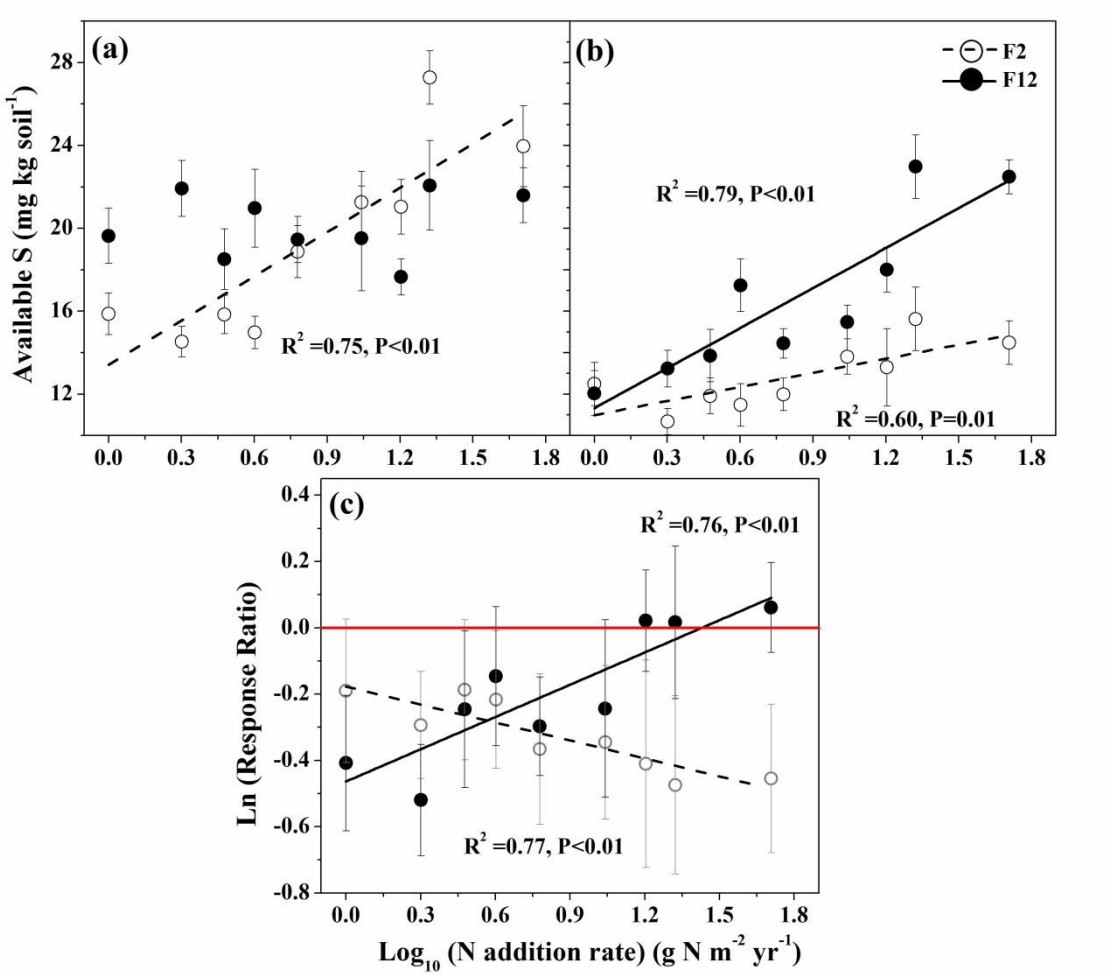

Figure 3 Effects of N addition intensity and frequency on soil available S concentration in unmown (a) and mown plots (b) and response ratio of soil available S to mowing practice along the N addition rate (c). Dashed and solid regression lines represent 2 and 12 N additions year^{-1}, respectively. Error bars indicate standard errors in figures (a) and (b), and 95% confidence intervals in figure (c).

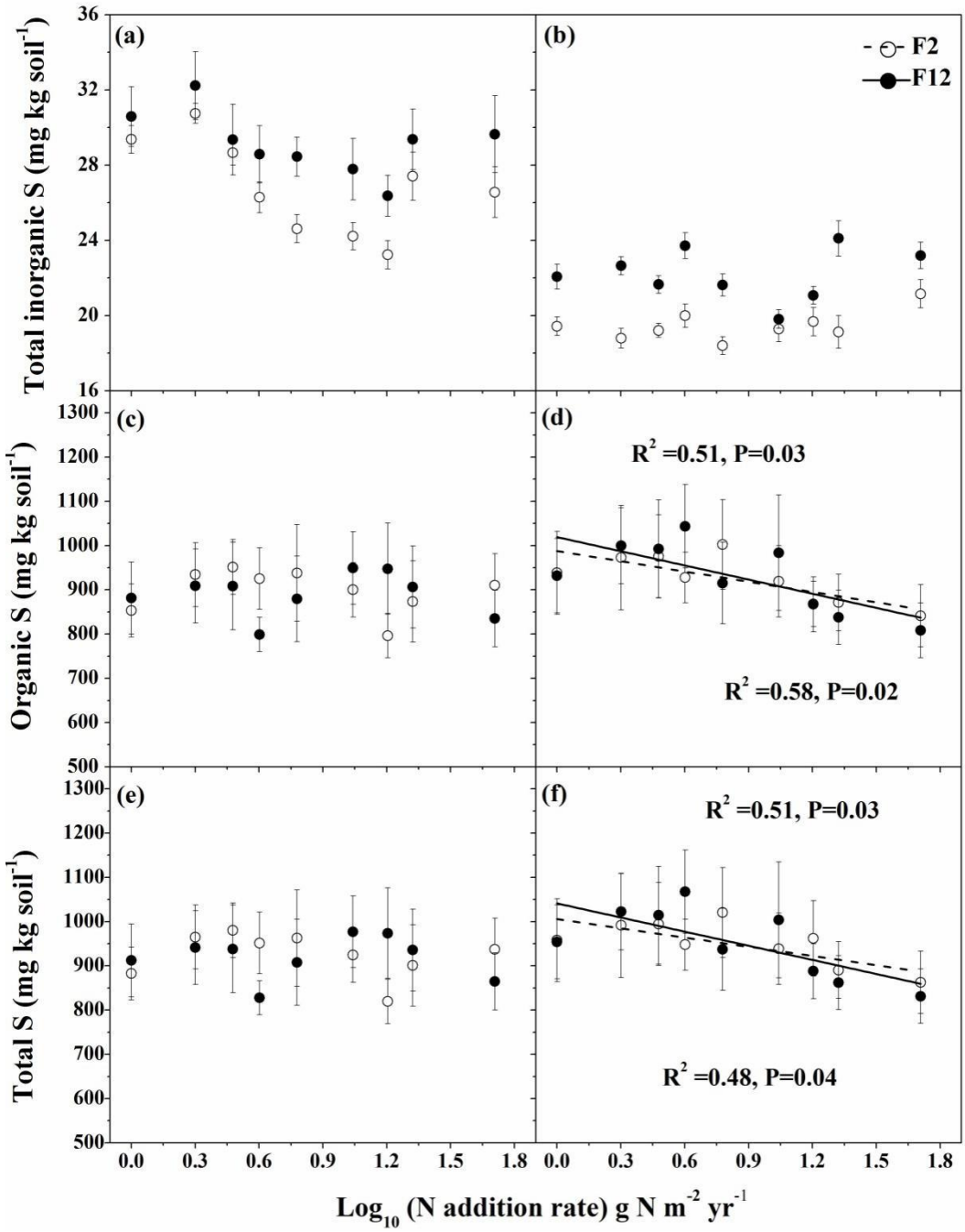

Figure 4 Effects of N addition intensity and frequency on concentrations of soil total inorganic S (a, b), organic S (c,d) and total S (e, f) in unmown (left) and mown (right) plots. Dashed and solid regression lines correspond to 2 and 12 N additions year^{-1}, respectively. Error bars indicate standard errors.

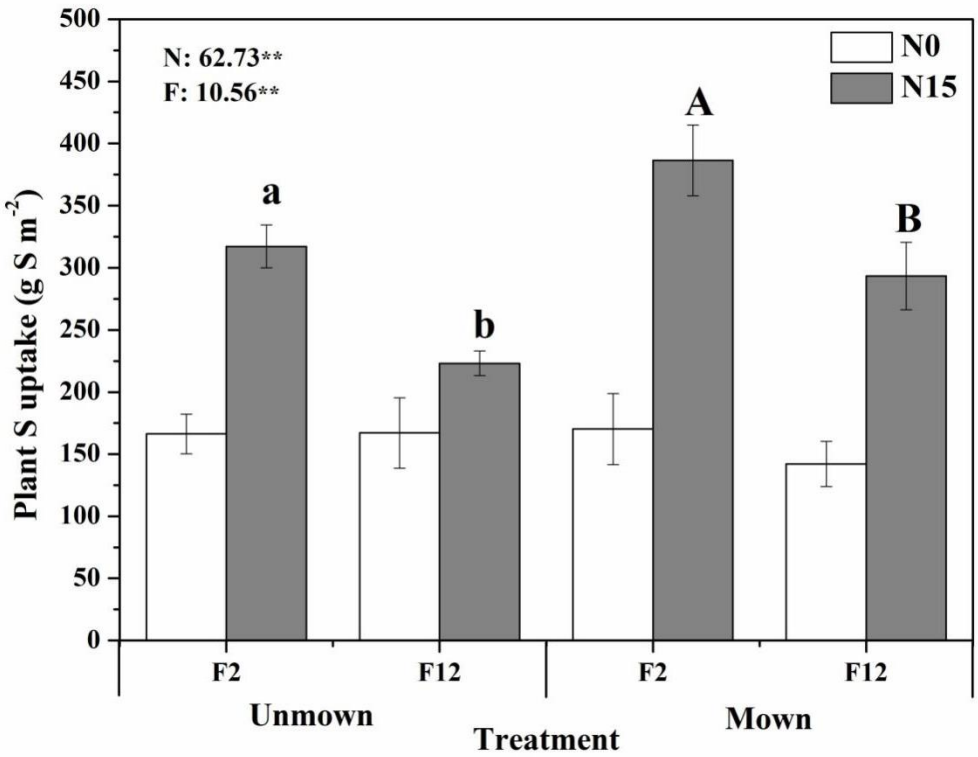

Figure 5 Effect of N addition intensity and frequency on plant S uptake by dominant species of *Leymus chinensis* and *Stipa grandis* without and with mowing (only plant samples in 0 and 15 g N m^{-2} $year^{-1}$ were measured). F2 and F12 indicate low and high frequency of N addition, respectively. Error bars indicate standard error. Effects of N addition intensity (N) and frequency (F) from three-way ANOVAs were labeled with ** representing significance level at 0.01. Different letters above the bars represent significant difference among means for the N addition frequency (F2 *vs.* F12) at 15 g N m^{-2} yr^{-1} (N15) without (lowercase letters) and with mowing (capital letters) separately. No significant difference was detected (not labeled) between the two N frequencies at 0 g N m^{-2} yr^{-1} for both mown and unmown treatments (N0).

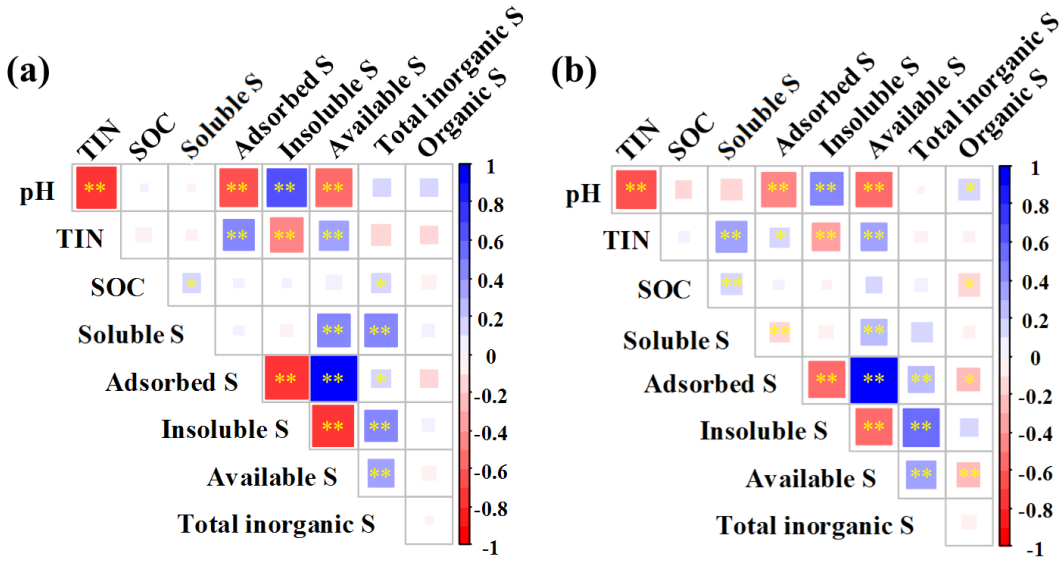

Figure 6 Correlations between soil parameters and S fractions with low (a) and high (b) frequency of N addition across N addition intensity and mowing treatments. Changing color from blue to red indicates correlations from positive to negative as exhibited in bars at right side of each panel. * and ** represent significance level at 0.05 and 0.01, respectively.

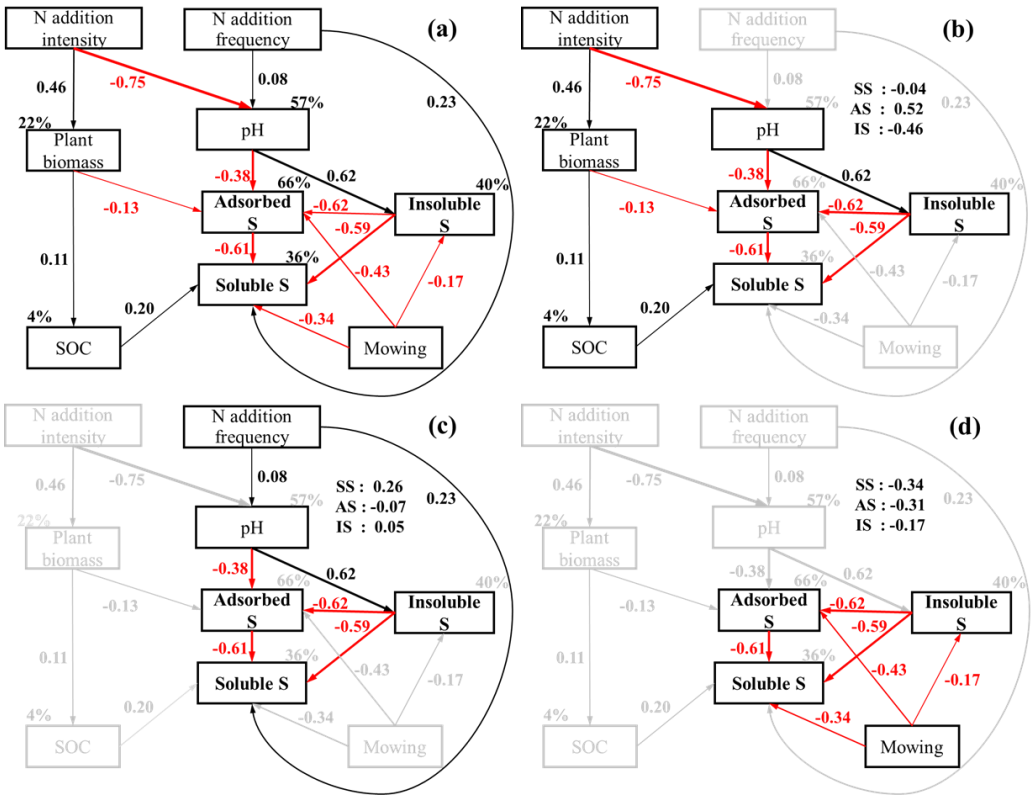

Figure 7 Structural equation modeling (SEM) illustrating the pathways of effects of N addition intensity, frequency and mowing on soil parameters and inorganic S fractions. Panel (a) shows the original model; panel (b) depicts the pathways related to effects of N intensity; panel (c) depicts the pathways related to effects of N frequency; and panel (d) depicts the depicts the pathways related to effects of mowing. Arrows indicate significantly positive (black) and negative (red) effects with the effect size proportional to arrow width. Numbers adjacent to arrows were standardized path coefficients, and percentages close to endogenous variables indicated the variance explained by the model (R^2). The numbers in the top-right corner denote the standardized total effects of N intensity, frequency and mowing on soluble S (SS), adsorbed S (AS) and insoluble S (IS), respectively. The final SEM fitted the data well as suggested by the fitting parameters (χ^2= 21.06, df = 23, P = 0.58, RMSEA= 0.000, AIC=85.06).