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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

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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 ecosystem 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 (water-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. Soil water-soluble and adsorbed S concentrations significantly increased, while insoluble S decreased with increasing intensity of N input. Such changes were correlated with soil pH and total inorganic nitrogen (TIN) concentration. High frequency of N addition increased the concentrations of water-soluble S, adsorbed S and available S as compared to low frequency of N addition in mown plots. Mowing significantly decreased all soil inorganic S fractions by reducing S replenishment via plant residue return. Mowing significantly interacted with both N addition intensity and frequency to affect inorganic S fractions, in that adsorbed S and available S showed no response to N addition intensity in unmown plots but significantly increased in mown plots under high N frequency. Mowing interacted with N addition intensity to decrease soil S pool size, suggesting that

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biomass removal under N input would cause soil S depletion in this temperate grassland. Nitrogen addition could 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. 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 on soil S availability in semi-arid grasslands.

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

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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 SO_4^{2-} (including water-soluble SO_4^{2-} and adsorbed SO_4^{2-}) and insoluble SO_4^{2-} coprecipitated with $CaCO_3$ (Fig. 1). Changes in the soil inorganic S pool play a major role in S dynamics because it can impact the transformation of soil organic S and plant S uptake (McGill and Cole, 1981; Ghani et al., 1993; 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 ecosystem 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 also have negative effects on S cycling rate due to the inhabitation 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 addition, 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 in either dry or wet form (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 frequency of N input influence transformation of soil inorganic S fractions still remains largely unknown.

Mowing is a common management practice in temperate grasslands (Giese et al., 2013), 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 influencing 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

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role in global S cycle. Primary productivity of temperate grasslands is mainly limited by N availability and typically sensitive to N addition (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 (water-soluble S and adsorbed S) concentration by promoting insoluble S dissolution with drop of soil pH 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 °C, varying from -21.4 °C in January to 19.7 °C 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 fertilizers were 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} yr^{-1}) crossed with two N addition frequencies (2 times yr^{-1} *vs.* 12 times yr^{-1}) and two mowing regimes (unmowing and mowing). 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

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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 °C for 48 h. Annual mowing was conducted at the end of August using a hay mower at 10-cm height to simulate the overgrazing and 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 °C 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). 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 $FeSO_4$. Soil total inorganic nitrogen (TIN) concentration was calculated as the sum of ammonium and nitrite, 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).

All the S fractions were extracted at 5:1 (w/v) water: soil ratio and quantified by turbidimetry with 0.5g of $BaCl_2$ crystals at 440nm using a UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan) (Tabatabai and Bremner, 1972). Briefly, 5.0 g of the air-dried soil was mixed with 25ml extractant (0.01*M* $Ca(H_2PO_4)_2$ for available S, 0.01 *M* $CaCl_2$ for water-soluble S and 1 *M* HCl for insoluble S) (Roberts and Bettany, 1985) and shaken at 400 rpm for 60 min at 25 °C. The extracts were then filtered and digested with 1ml of H_2O_2 for 20 min to decompose organic matter. The cooled solutions were mixed with 0.5ml of HCl (1:4) and 1 ml of acacia solution successively

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and adjusted to 25 ml. The concentration of sulfur in the mixture was determined by turbidimetry. Adsorbed sulfur, total inorganic sulfur and organic sulfur concentrations were calculated as follows: adsorbed S = available S – water-soluble S, total inorganic S = available S + insoluble S, and organic S = total S – total inorganic S.

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°C. 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:

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

where S_i represents S concentration in plants 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 each S fraction in total inorganic S was calculated and Duncan's HSD post-hoc 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:

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

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. 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 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.

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3 Results

3.1 Effects of N addition and mowing on soil characters

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 intensity 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 water-soluble S

Mowing significantly decreased soil water-soluble S concentration by up to 47% at the two N frequencies (Fig. 2a, b; Table 2). High frequency of N addition significantly increased water-soluble S concentration by up to 90% with both unmowing and mowing practices, resulting in a significant M×F effect (Fig. 2a, b; Table 2). At high frequency of N addition, intensity of N addition increased water-soluble S for both unmown (Fig. 2a, P=0.004) and mown plots (Fig. 1b, 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, intensity of N addition 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 55% irrespective of both intensity and frequency of N addition (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, $P = 0.02$ 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×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% 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% (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 increased the negative effect of mowing on soil available S concentration at low frequency of N addition (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 fraction (sum of water-soluble and adsorbed S) responded differently to N addition and mowing (Fig. 4). For unmown plots, high intensity of N addition enhanced the proportion of available S at low frequency of N addition (Fig. 4a, from 43.2 to 64.7%), while it showed no impact at high frequency of N addition (Fig. 4b). In contrast, soil available S proportion increased with increasing N addition intensity at high instead of low frequency of N addition for mown plots (Fig. 4c *vs.* 4d).

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

Effects of mowing, frequency of N addition, and intensity of N addition were significant on soil total inorganic S (Table 2). Mowing significantly decreased total inorganic S at both low and high frequency of N addition by up to 53% (Fig. 5a, b). In mown plots, high frequency of N addition significantly increased soil total inorganic S concentration by as much as 30% comparing to low N frequency (Fig. 5b; Table 2).

Nitrogen addition increased soil total inorganic S along the N gradient at both low (from 19.7 to 25.4 mg kg soil^{-1}, P=0.01) and high N addition frequency (from 24.0 to 31.2 mg kg soil^{-1}) of mown plots (Fig. 5b). Soil total S concentration was not affected by intensity of N addition, frequency of N addition, or mowing treatments (Table 2). However, the mean value of soil total S concentration tended to decrease with increasing N addition intensity as suggested by its linear correlation with N addition intensity at both low (P = 0.04) and high (P = 0.03) N addition frequency in mown plots (Fig. 5d).

High intensity of nitrogen addition increased aboveground biomass regardless of N addition frequencies and mowing management (Fig. S1). 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. 6). Nitrogen addition intensity increased plant S concentration of two dominant species (Fig. S2) and relative biomass proportion of *Stipa grandis* (Fig. S3).

3.5 Relationships between soil characters and S fractions

Soil pH was negatively correlated with adsorbed S, available S and total inorganic S concentrations under both low (Fig. 7a) and high frequency of N addition (Fig. 7b) across intensity of N addition and mowing treatments (all $P < 0.01$). However, insoluble S was positively correlated with pH only under low frequency of N addition ($P<0.01$, Fig. 7a). Soil TIN was positively correlated with adsorbed S and available S under both low and high frequency of N addition ($P < 0.05$, Fig. 7a, b). Soil organic carbon was positively correlated with water-soluble S only in high N frequency plots. Under high N frequency, water-soluble S was negatively correlated with adsorbed S and insoluble S concentrations, and adsorbed S was positively correlated with insoluble S concentration ($P < 0.01$). Organic S concentration was negatively correlated with adsorbed S, available S and total inorganic S concentrations at high frequency of N addition (Fig. 7b).

Results of SEM showed that both N addition intensity and mowing practice had direct and indirect effects on soil S fractions under both N frequencies (Fig. 7c, d). Nitrogen addition intensity had a significantly direct and negative effect (standardized effect size: -0.17) on insoluble S at low N frequency (Fig. 7c), and it was an indirect effect (standardized effect size: -0.26) by altering TIN at high N frequency (Fig. 7d). Nitrogen addition intensity only had indirectly positive effect on adsorbed S (standardized effect size: 0.47) at low N addition frequency, while it showed both direct and indirect effects at high N frequency (Fig. 7c, d). Nitrogen addition intensity had indirect and negative effect on organic S concentration by altering pH at both N addition frequencies. Mowing had directly negative effects on soil insoluble S, adsorbed S and water-soluble S concentrations at both N frequencies, with stronger effect sizes at low frequency of N addition (standardized effect size:-0.61, -0.35 and -0.98 *vs.* -0.58, not significant and -0.60, respectively). Water-soluble S concentration was directly and negatively affected by both adsorbed and insoluble S at both N frequencies (Fig. 7a, b).

4 Discussion

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

As expected, increase of water-soluble S was partially due to the dissolution of soil insoluble S with increasing N addition intensity. Water-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). Under natural conditions, free sulfate in soil could also 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, Mg and Na (Wang et al., 2018). However, under excessive N input, insoluble-S dissolution could sequentially enhance SO_4^{2-} mobility as affected by soil acidification. This postulation was further confirmed by the significant relationships of soil pH with insoluble S (positive) at low N frequency and with available S concentrations (negative) at both N frequencies (Fig. 7a,b).

In this study, higher adsorbed S under N addition was mainly derived from higher ability of sulfate adsorption with decrease of soil pH, which was in line with Nodvin et al. (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).

Nitrogen addition potentially increased organic S mineralization as indicated by the increased total inorganic S but unchanged (unmown plots) or even decreased (mown plots) total S concentration. This was consistent with previous observations where N addition enhanced mineralization of organic S to increase S availability by elevating microbial activity (Ghani et al., 1992). Soil N availability would also have considerable impacts on the mineralization of organic S (Gharmakher et al., 2009). The increases of soil TIN following N input possibly accelerated organic S mineralization in this study. Moreover, higher plant S uptake under N input (Fig. 6) could promote biochemical mineralization (McGill and Cole, 1981).

4.2 Effects of N addition frequency on soil inorganic S fractions

Partially contrary to our second hypothesis, low frequency of N addition decreased water-soluble S, available S (only in mown plots) and total inorganic S (mown plots) concentrations as compared to high frequency (Fig. 2a, b, d; Fig. 5b). Soil available S concentration was mainly determined by the input from dissolution of insoluble S and output to plant uptake and leaching. There is a sharper pH decrease with increasing N intensity under low frequency of N addition, as compared to that under high frequency of N addition (Ning et al., 2015; Wang et al., 2018). In this study, lower soil pH decreased insoluble S concentration by promoting its dissolution (Fig. 2e) and increased S adsorption (Fig. 2d) in unmown plots at low frequency of N addition. Therefore, lower frequency of N addition stimulated the transformation of insoluble S into adsorbed S. Another potential explanation could be that large-pulse water input resulted in higher leaching loss of water-soluble S during N addition at low frequency than the high N frequency treatment by adding small-amount water each time. Indeed, infrequent and extreme rainfall pulses have been found to increase SO_4^{2-} leaching in sandy soil (Eriksen and Askegaard, 2000). Moreover, plant biomass and plant S uptake were promoted by large dose of N application at low frequency of N addition (Fig. 6 and S1), which could potentially stimulate the dissolution of insoluble S and mineralization of organic S (Hu et al., 2002; McGill and Cole, 1981). Therefore, significantly lower water-soluble S and available S could be a result of higher amount of S output from plant retention and leaching than input from insoluble S dissolution under lower frequency of N addition.

Negative correlation between water-soluble S and adsorbed/ insoluble S concentrations (Fig. 7b) indicated the complementary roles of these fractions under high frequency of N addition. However, at lower frequency of N addition, weak relationships among soil S fractions (including inorganic and organic S, Fig. 7a) suggested that intense S output pathways broken the coupling of inorganic S fractions.

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This was further confirmed by fewer influencing pathways between S fractions in the SEM at low N frequency than the high frequency (Fig. 7c *vs.* Fig. 7d). Under low frequency of N addition, weak relationships among S fractions could be also attributed to unchanged proportion of water-soluble S (Fig. 4a) due to the trade-off between its input (i.e. dissolution and mineralization) and output (i.e. adsorption and uptake) processes.

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). Without N addition, mowing showed no impact on the rate of inorganic S transformations as suggested by the unchanged proportion of available S *vs*. insoluble S in spite of the decrease in concentrations of inorganic S fractions (Fig. 4 and 5). Unaffected proportion of inorganic S fractions suggested that biomass removal alone did not stimulate the transformation of S fractions from unavailable forms to available ones. This could probably due to replenishment of inorganic S fractions from organic S mineralization with the presence of relatively low plant S uptake by dominant species without N addition (Fig. 6).

However, when N was added, negative mowing effect on soil available S was

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suggested to be exacerbated due to enhanced plant S uptake coincident with higher plant biomass (Jackson, 2000). This was only the case at low frequency of N addition in this study, but high N frequency alleviated negative mowing effect and even turned 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. Evidence from SEM also supported this speculation, where direct and negative effects of mowing on all inorganic S fractions were lower at high frequency of N addition than the low frequency (Fig. 7). This could be due to higher intensity of plant S uptake promoted inorganic S transformation and strengthened the relationship between S fractions at lower frequency of N addition (Fig. 6).

Mowing interacted with N addition increased S uptake by dominant species (Fig. 5) which was probably due to higher S concentration in *Stipa grandis* (Fig. S2) and increases of biomass proportion of *Stipa grandis* after mowing (Fig. S3). Therefore, the increase of S removal from the grassland ecosystem could potentially stimulate available S formation via abiotic dissolution of insoluble S and organic S mineralization. It was reasonable to detect the decrease of soil total S and insoluble S but increase of water-soluble S and adsorbed S concentrations under N addition and mowing treatment.

5 Conclusions

Increasing the intensity of N input enhanced soil available S fractions through directly and indirectly affecting soil pH, TIN 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 water-soluble S, adsorbed S and available S concentrations under mowing treatment. Mowing should be considered as an essential factor in regulating the effects of N addition intensity and frequency on soil S dynamics in semi-arid grassland ecosystems. The study could provide insights for sustainable grassland management in terms of fertilization and mowing practices by concerning their influences on ecosystem S cycling.

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References

Aneja, V. P., Roelle, P. A., Murray, G. C., Southerland, J., Erisman, J. W., Fowler, D., Asman, W. A. H., and Patni, N.: Atmospheric nitrogen compounds II: emissions, transport, transformation, deposition and assessment, Atmos. Environ., 35, 1903-1911, https://doi.org/10.1016/S1352-2310(00)00543-4, 2001.

Barrow, N. J.: A comparison of the mineralization of nitrogen and of sulphur from decomposing organic materials, Aust. J. Agric. Res., 11, 960-969, https://doi.org/10.1071/AR9600960, 1960.

Barton, L., Wan, G. G. Y., Buck, R. P., and Colmer, T. D.: Does N fertiliser regime influence N leaching and quality of different-aged turfgrass (Pennisetum clandestinum) stands?, Plant Soil, 316, 81-96, https://doi.org/10.1007/s11104-008-9761-7, 2008.

Bilbrough, C. J. and Caldwell, M. M.: Exploitation of springtime ephemeral N pulses by six great basin plant species, Ecology, 78, 231-243, https://doi.org/10.2307/2265992, 1997.

Blum, S. C., Lehmann, J., Solomon, D., Caires, E. F., and Alleoni, L. R. F.: Sulfur forms in organic substrates affecting S mineralization in soil, Geoderma, 200, 156-164, https://doi.org/10.1016/j.geoderma.2013.02.003, 2013.

Chen, H., Yang, L., Wen, L., Luo, P., Liu, L., Yang, Y., Wang, K., and Li, D.: Effects of nitrogen deposition on soil sulfur cycling, Global Biogeochem. Cycles, 30, 1568-1577, https://doi.org/10.1002/2016gb005423, 2016.

Cheng, X., Luo, Y., Su, B., Verburg, P. S. J., Hui, D., Obrist, D., Arnone, J. A., Johnson, D. W., and Evans, R. D.: Responses of net ecosystem CO_2 exchange to nitrogen fertilization in experimentally manipulated grassland ecosystems, Agric. For. Meteorol., 149, 1956-1963, https://doi.org/10.1016/j.agrformet.2009.07.001, 2009.

Chiang, H. C., Lo, J. C., and Yeh, K. C.: Genes associated with heavy metal tolerance and accumulation in Zn/Cd hyperaccumulator Arabidopsis halleri: a genomic survey with cDNA microarray, Environ. Sci. Technol., 40, 6792-6798, https://doi.org/10.1021/es061432y, 2006.

De Bona, F. D. and Monteiro, F. A.: Nitrogen and sulfur fertilization and dynamics in a Brazilian Entisol under pasture, Soil Sci. Soc. Am. J., 74, 1248-1258, https://doi.org/10.2136/sssaj2009.0228, 2010.

Dentener, F., Drevet, J., Lamarque, J. F., Bey, I., Eickhout, B., Fiore, A. M., Hauglustaine, D., Horowitz, L. W., Krol, M., Kulshrestha, U. C., Lawrence, M., Galy-Lacaux, C., Rast, S., Shindell, D., Stevenson, D., Van Noije, T., Atherton, C., Bell, N., Bergman, D., Butler, T., Cofala, J., Collins, B., Doherty, R., Ellingsen, K., Galloway, J., Gauss, M., Montanaro, V., Müller, J. F., Pitari, G., Rodriguez, J., Sanderson, M., Solmon, F., Strahan, S., Schultz, M., Sudo, K., Szopa, S., and Wild, O.: Nitrogen and sulfur deposition on regional and global scales: A multimodel evaluation, Global Biogeochem. Cycles, 20, GB4003, 1-21, https://doi.org/10.1029/2005GB002672, 2006.

Eriksen, J. and Askegaard, M.: Sulphate leaching in an organic crop rotation on sandy soil in Denmark, Agri. Ecosyst. Environ. 78, 107-114, https://doi.org/10.1016/S0167-8809(99)00117-6, 2000.

Feechan, A., Kwon, E., Yun, B. W., Wang, Y., Pallas, J. A., and Loake, G. J.: A central role for S-nitrosothiols in plant disease resistance, Proc. Natl. Acad. Sci. U.S.A., 102, 8054-8059, https://doi.org/10.1073/pnas.0501456102, 2005.

Ghani, A., McLaren, R., and Swift, R.: Sulphur mineralisation and transformations in soils as influenced by additions of carbon, nitrogen and sulphur, Soil Biol. Biochem., 24, 331-341, https://doi.org/10.1016/0038-0717(92)90193-2,1992.

Ghani, A., Mclaren, R. G., and Swift, R. S.: The incorporation and transformation of ^{35}S in soil: effects of soil conditioning and glucose or sulphate additions, Soil Biol. Biochem., 25, 327-335, https://doi.org/,10.1016/0038-0717(93)90131-T, 1993.

Giese, M., Brueck, H., Gao, Y., Lin, S., Steffens, M., xf, gel-Knabner, I., Glindemann, T., Susenbeth, A., Taube, F., Butterbach-Bahl, K., Zheng, X., Hoffmann, C., Bai, Y., and Han, X.: N balance and cycling of Inner Mongolia typical steppe: a comprehensive case study of grazing effects, Ecol. Monogr., 83, 195-219, https://doi.org/10.1890/12-0114.1, 2013.

Hao, Y., Kang, X., Wu, X., Cui, X., Liu, W., Zhang, H., Li, Y., Wang, Y., Xu, Z., and Zhao, H.: Is frequency or amount of precipitation more important in controlling CO_2 fluxes in the 30-year-old fenced and the moderately grazed temperate steppe?, Agric. Ecosyst. Environ., 17, 63-71,

Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-526
Manuscript under review for journal Biogeosciences
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https://doi.org/10.1016/j.agee.2013.03.011, 2013.

Harpole, W. S., Potts, D. L., and Suding, K. N.: Ecosystem responses to water and nitrogen amendment in a California grassland, Global Change Boil., 13, 2341-2348, https://doi.org/10.1111/j.1365-2486.2007.01447.x, 2007.

Hu, Z., Yang, Z., Xu, C., Haneklaus, S., Cao, Z., and Schnug, E.: Effect of crop growth on the distribution and mineralization of soil sulfur fractions in the rhizosphere, J. Plant Nutr. Soil Sci., 165, 249-254, https://doi.org/10.1002/1522-2624(200206)165:3<249::AID-JPLN1111249>3.0.CO;2-N, 2002.

Jackson, G. D.: Effects of nitrogen and sulfur on canola yield and nutrient uptake, Agron. J., 92, 644-649, https://doi.org/10.2134/agronj2000.924644x, 2000.

Janzen, H. H. and Ellert, B. H.: Sulfur dynamics in cultivated temperate agroecosystems. In: Sulphur in the Environment, Maynard, D.G. (Ed.), Marcel Dekker, New York, 11-43, 1998.

Johnson, D. W. and Todd, D.: Relationships among iron, aluminum, carbon, and sulfate in a variety of forest soils 1, Soil Sci. Soc. Am. J., 47, 792-800, https://doi.org/ 10.2136/sssaj1983.03615995004700040035x, 1983.

Juszczuk, I. M. and Ostaszewska, M.: Respiratory activity, energy and redox status in sulphur-deficient bean plants, Environ. Exp. Bot., 74, 245-254, https://doi.org/10.1016/j.envexpbot.2011.06.006, 2011.

Kertesz, M. A. and Mirleau, P.: The role of soil microbes in plant sulphur nutrition, J.

Exp. Bot., 55, 1939-1945, https://doi.org/10.1093/jxb/erh176, 2004.

Koncz, P., Balogh, J., Papp, M., Hidy, D., Pintér, K., Fóti, S., Klumpp, K., and Nagy, Z.: Higher soil respiration under mowing than under grazing explained by biomass differences, Nutr. Cycling Agroecosyst., 103, 201-215, https://doi.org/10.1007/s10705-015-9732-3, 2015.

Kost, D., Chen, L., and Dick, W. A.: Predicting plant sulfur deficiency in soils: results from Ohio, Biol. Fertil. Soils, 44, 1091-1098, https://doi.org/10.1007/s00374-008-0298-y, 2008.

Liu, X., Zhang, Y., Han, W., Tang, A., Shen, J., Cui, Z., Vitousek, P., Erisman, J. W., Goulding, K., Christie, P., Fangmeier, A., and Zhang, F.: Enhanced nitrogen deposition over China, Nature, 494, 459-462, https://doi.org/10.1038/nature11917, 2013.

Lü, X., Lü, F., Zhou, L., Han, X., and Han, X.: Stoichiometric response of dominant grasses to fire and mowing in a semi-arid grassland, J. Arid Environ., 78, 154-160, https://doi.org/ 10.1016/j.jaridenv.2011.11.008, 2012.

McGill, W. B. and Cole, C. V.: Comparative aspects of cycling of organic C, N, S and P through soil organic matter, Geoderma, 26, 267-286, https://doi.org/10.1016/0016-7061(81)90024-0, 1981.

Niknahad Gharmakher, H., Machet, J. M., Beaudoin, N., and Recous, S.: Estimation of sulfur mineralization and relationships with nitrogen and carbon in soils, Biol. Fertil. Soils, 45, 297-304, https://doi.org/10.1007/s00374-008-0332-0, 2009.

Ning, Q., Gu, Q., Shen, J., Lü, X., Yang, J., Zhang, X., He, J., Huang, J., Wang, H., Xu, Z., and Han, X.: Effects of nitrogen deposition rates and frequencies on the abundance of soil nitrogen-related functional genes in temperate grassland of northern China, J. Soils Sediments, 15, 694-704, https://doi.org/10.1007/s11368-015-1061-2, 2015.

Niu, S., Wu, M., Han, Y. I., Xia, J., Zhang, Z. H. E., Yang, H., and Wan, S.: Nitrogen effects on net ecosystem carbon exchange in a temperate steppe, Global Change Boil., 16, 144-155, https://doi.org/10.1111/j.1365-2486.2009.01894.x, 2010.

Nodvin, S. C., Driscoll, C. T., and Likens, G. E.: The effect of pH on sulfate adsorption by a forest soil, Soil Sci., 142, 69-75, https://doi.org/10.1097/00010694-198608000-00002, 1986.

Phoenix, G. K., Emmett, B. A., Britton, A. J., Caporn, S. J. M., Dise, N. B., Helliwell, R., Jones, L., Leake, J. R., Leith, I. D., Sheppard, L. J., Sowerby, A., Pilkington, M. G., Rowe, E. C., Ashmore, M. R., and Power, S. A.: Impacts of atmospheric nitrogen deposition: responses of multiple plant and soil parameters across contrasting ecosystems in long-term field experiments, Global Change Boil., 18, 1197-1215, https://doi.org/, 2012.

Roberts, T. L. and Bettany, J. R.: The influence of topography on the nature and distribution of soil sulfur across a narrow environmental gradient, Can. J. Soil Sci., 65, 419-434, https://doi.org/10.4141/cjss85-046, 1985.

Scherer, H.W., Welp, G., and Förster, S.: Sulfur fractions in particle-size separates as influenced by long-term application of mineral and organic fertilizers, Plant, Soil Environ., 58, 242-248, https://doi.org/10.17221/78/2012-PSE, 2012.

Scherer, H.W.: Sulfur in soils, J. Plant Nutr. Soil Sci., 172, 326-335, https://doi.org/10.1002/jpln.200900037, 2009.

Smith, M. D., Knapp, A. K., and Collins, S. L.: A framework for assessing ecosystem dynamics in response to chronic resource alterations induced by global change, Ecology, 90, 3279-3289, https://doi.org/10.2307/25660976, 2009.

Solomon, D., Lehmann, J., Tekalign, M., Fritzsche, F., and Zech, W.: Sulfur fractions in particle-size separates of the sub-humid Ethiopian highlands as influenced by land use changes, Geoderma, 102, 41-59, https://doi.org/10.1016/s0016-7061(00)00103-8, 2001.

Tabatabai, M. A. and Bremner, J. M.: Forms of sulfur, carbon, nitrogen and sulfur relationships in Iowa soils, Soil Sci., 114, 380-386, https://doi.org/10.1097/00010694-197211000-00009, 1972.

Tabatabai, M. A.: Chemistry of Sulfur in Soils. In: Chemical Processes in Soils, Tabatabai, M. A. and Sparks, D. L. (Eds.), SSSA Book Series, 8, Soil Science Society of America, Madison, WI, 2005.

Tisdale, S., Nelson, W., Beaton, J., and Havlin, J.: Soil Fertility and Fertilizers. Prentice Hall, New Jersey, 1993.

Wang, C., Butterbach-Bahl, K., He, N., Wang, Q., Xing, X., and Han, X.: Nitrogen addition and mowing affect microbial nitrogen transformations in a C4 grassland in northern China, Eur. J. Soil Sci., 66, 485-495, https://doi.org/10.1111/ejss.12231, 2015.

Wang, R., Creamer, C. A., Wang, X., He, P., Xu, Z. W., and Jiang, Y.: The effects of a 9-year nitrogen and water addition on soil aggregate phosphorus and sulfur availability in a semi-arid grassland, Ecol. Indic., 61, 806-814, https://doi.org/10.1016/j.ecolind.2015.10.033, 2016.

Wang, R., Zhang, Y., He, P., Yin, J., Yang, J., Liu, H., Cai, J., Shi, Z., Feng, X., Dijkstra, F. A., Han, X., and Jiang, Y.: Intensity and frequency of nitrogen addition alter soil chemical properties depending on mowing management in a temperate steppe, J. Environ. Manage., 224, 77-86, https://doi.org/10.1016/j.jenvman.2018.07.036, 2018.

White, R. P., Murray, S. and Rohweder, M.: Pilot analysis of global ecosystems: grassland ecosystems, World Resources Institute, Washington DC, 2000.

Wulff-Zottele, C., Gatzke, N., Kopka, J., Orellana, A., Hoefgen, R., Fisahn, J., and Hesse, H.: Photosynthesis and metabolism interact during acclimation of Arabidopsis thaliana to high irradiance and sulphur depletion, Plant, Cell Environ., 33, 1974-1988, https://doi.org/10.1111/j.1365-3040.2010.02199.x, 2010.

Yang, H., Jiang, L., Li, L., Li, A., Wu, M., and Wan, S.: Diversity-dependent stability under mowing and nutrient addition: evidence from a 7-year grassland experiment, Ecol. Lett., 15, 619-626, https://doi.org/10.1111/j.1461-0248.2012.01778.x, 2012.

Yang, Z., Singh, B. R., Hansen, S., Hu, Z., and Riley, H.: Aggregate associated sulfur fractions in long-Term (>80 Years) fertilized soils, Soil Sci. Soc. Am. J., 71, 163, https://doi.org/10.2136/sssaj2006.0242, 2007.

Zhang, Y., Han, X., He, N., Long, M., Huang, J., Zhang, G., Wang, Q., and Han, X.: Increase in ammonia volatilization from soil in response to N deposition in Inner Mongolia grasslands, Atmos. Environ., 84, 156-162, https://doi.org/ 10.1016/j.atmosenv.2013.11.052, 2014a.

Zhang, Y., Lu, X., Isbell, F., Stevens, C., Han, X., He, N., Zhang, G., Yu, Q., Huang, J., and Han, X.: Rapid plant species loss at high rates and at low frequency of N addition in temperate steppe, Global Change Boil., 20, 3520-3529, https://doi.org/10.1111/gcb.12611, 2014b.

Zhang, Y., Feng, J., Isbell, F., Lu, X., and Han, X.: Productivity depends more on the rate than the frequency of N addition in a temperate grassland, Sci. Rep., 5, 12558, https://doi.org/10.1038/srep12558, 2015.

Zhao, Y., Zhang, L., Chen, Y., Liu, X., Xu, W., Pan, Y., and Duan, L.: Atmospheric nitrogen deposition to China: A model analysis on nitrogen budget and critical load exceedance, Atmos. Environ., 153, 32-40, https://doi.org/10.1016/j.atmosenv.2017.01.018, 2017.

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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 rate (g N $m^{-2}year^{-1}$)	Unmown		Mown	
		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)ABbcd	7.01 (0.17)Ab
	15	6.24 (0.24)Bb	7.08 (0.18)Aa	6.42(0.30)ABcd	6.48 (0.18)ABc
	20	5.56 (0.16)c	6.22 (0.23)b	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)Ac	13.19(0.88)ABd	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)ABa	74.60 (6.22)Ba	111.94(15.14)Aa	71.93(10.32)Ba
SOC (%)	0	2.42 (0.06)ABa	2.17 (0.05)Cb	2.60 (0.09)Aa	2.22(0.11)BCab
	1	2.45 (0.08)a	2.24 (0.06)b	2.45 (0.09)ab	2.33 (0.15)ab
	2	2.17 (0.07)ABb	2.22 (0.06)ABb	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)Bbc	2.35(0.08)ABab
	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)Aa	2.33(0.08)ABab	2.10 (0.09)Bc	2.27(0.11)ABab
	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 and mowing practice 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

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(lowercase letters). F2 and F12 represent low and high frequency of N addition, respectively.

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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.

	Water-soluble S	Adsorbed S	Available S	Insoluble S	TIS	Total S
M	74.46**	60.40**	145.25**	231.36**	343.35**	2.91
F	120.82**	0.23	31.59**	1.85	25.22**	0.08
N	3.95**	11.36**	16.64**	12.62**	5.94**	1.48
M×F	4.80*	5.98*	12.59**	5.52*	1.31	0
M×N	1.22	0.84	0.66	3.69**	2.62**	0.80
F×N	5.05**	4.83**	2.53*	0.79	2.46*	0.39
M×F×N	1.45	6.65**	6.34**	1.40	1.54	1.42

Note: TIS represents soil total inorganic S;

* and ** represent significance levels ($P \leq 0.05$ and 0.01, respectively)

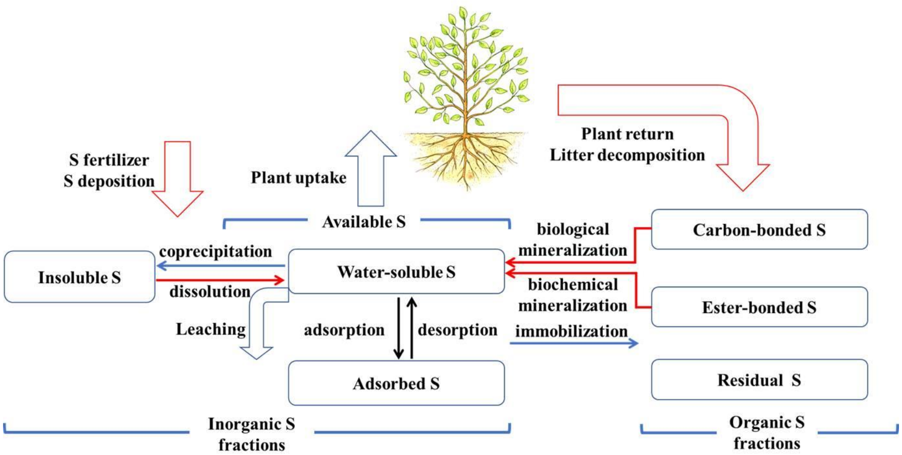

Figure 1 Conceptual scheme depicting the transformation of sulfur fractions in aerobic calcareous soils. Arrows indicate input (red) and output (blue) processes of soil total S (hollow) and available S (solid). Soil available S mainly occur as water-soluble (the most active fraction) and adsorbed S in aerobic soils. The adsorbed S is mainly controlled by reversible adsorption-desorption processes which are pH-dependent. Organic S can be mineralized into inorganic S through biological process (defined as microbial C oxidation of C-bonded S) and biochemical process (extracellular enzymatic hydrolysis of easter-bonded S).

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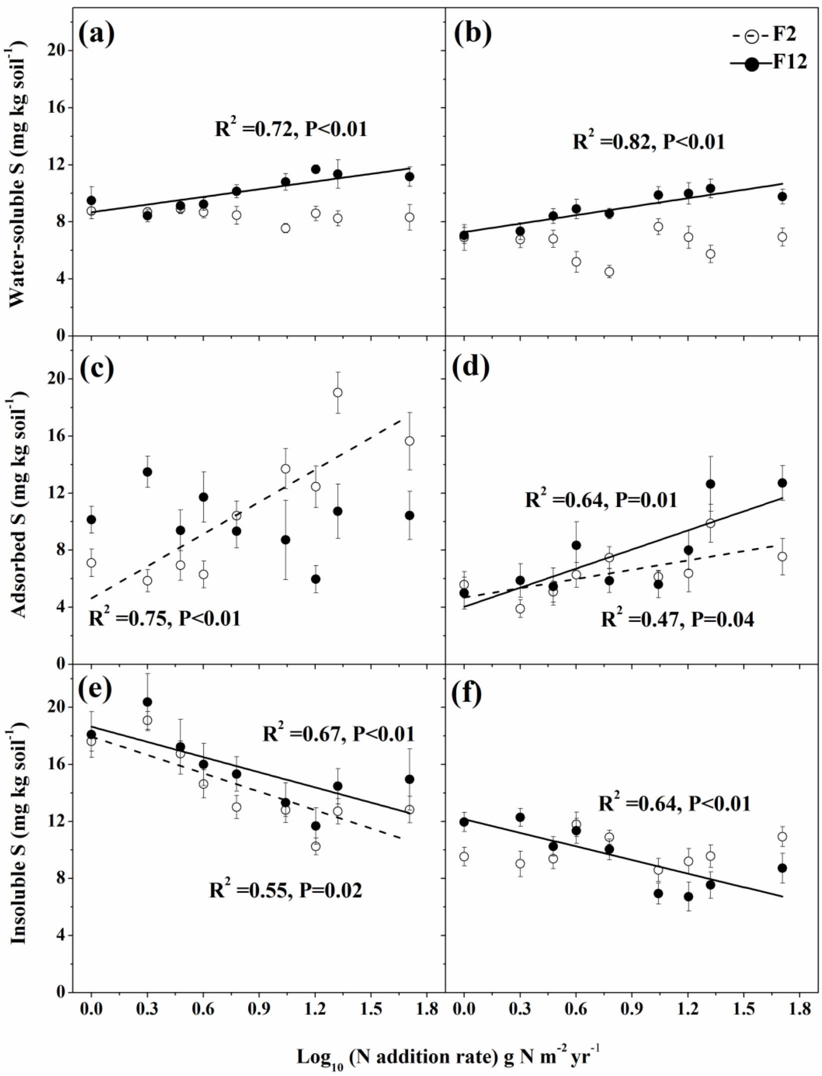

Figure 2 Effects of N addition intensity and frequency on concentrations of soil water-soluble S (a, b), adsorbed S (c, d) and insoluble S (e, f) in unmown (left figures) and mown plots (right figures). Dashed and solid regression lines represent 2 and 12 N additions year^{-1}, respectively. Error bars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

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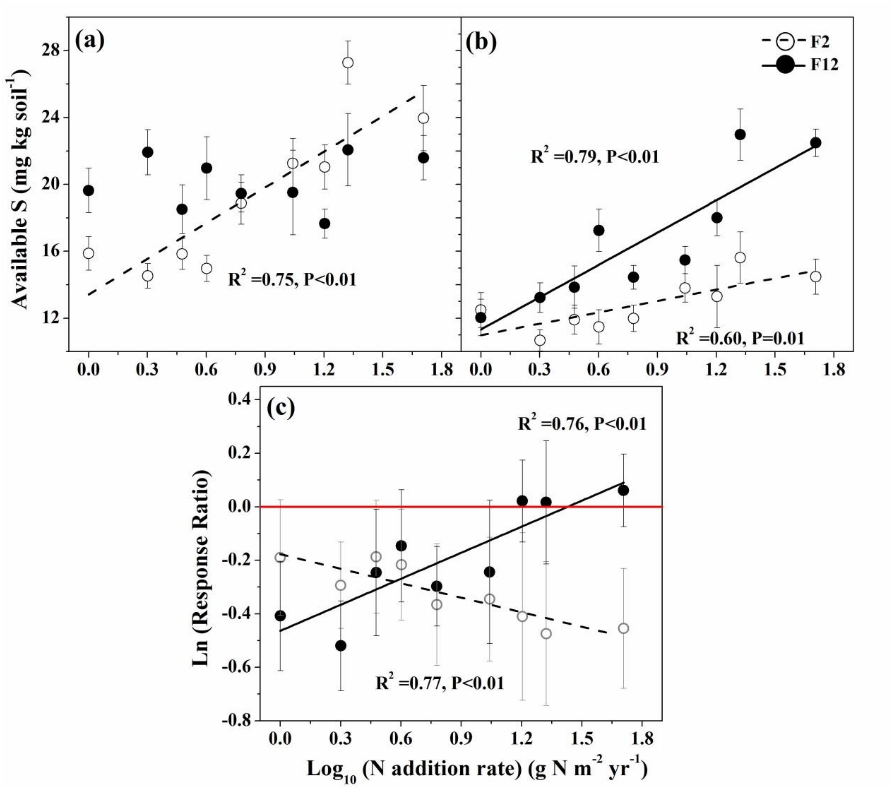

Figure 3 Effects of N addition intensity and frequency on soil available S concentration in unmown (a) and mown plots (b) and response ratio of soil available S to mowing practice along the N addition rate (c). Dashed and solid regression lines represent 2 and 12 N additions year^{-1}, respectively.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

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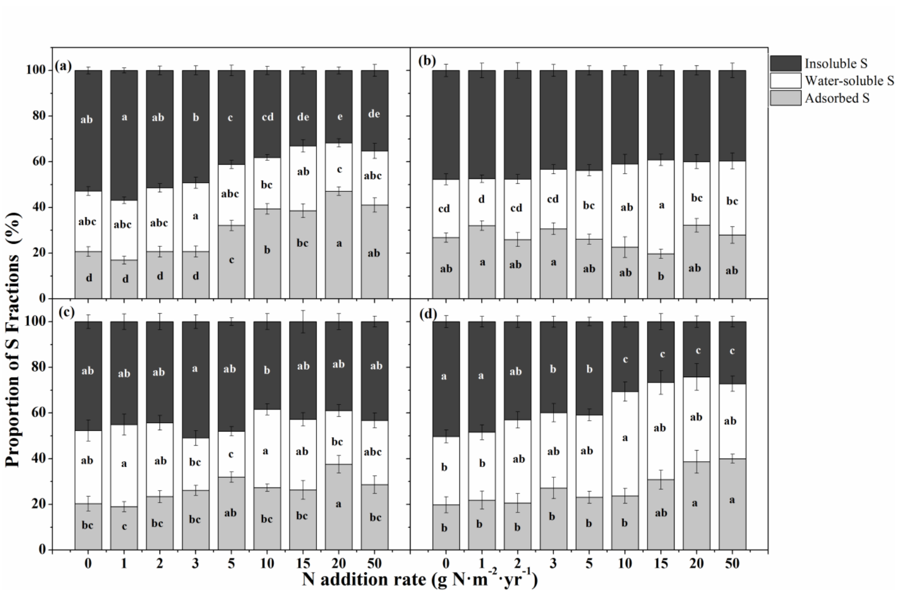

Figure 4 Proportion of inorganic sulfur fractions relative to total inorganic sulfur under low and high frequency of nitrogen (N) addition in unmown (a and b, respectively) and mown (c and d, respectively) plots. White, gray and black bars correspond to water-soluble S, adsorbed S and insoluble S, respectively. Error bars indicate standard errors. Different letters represent significant difference among N addition rates for each inorganic S fraction.

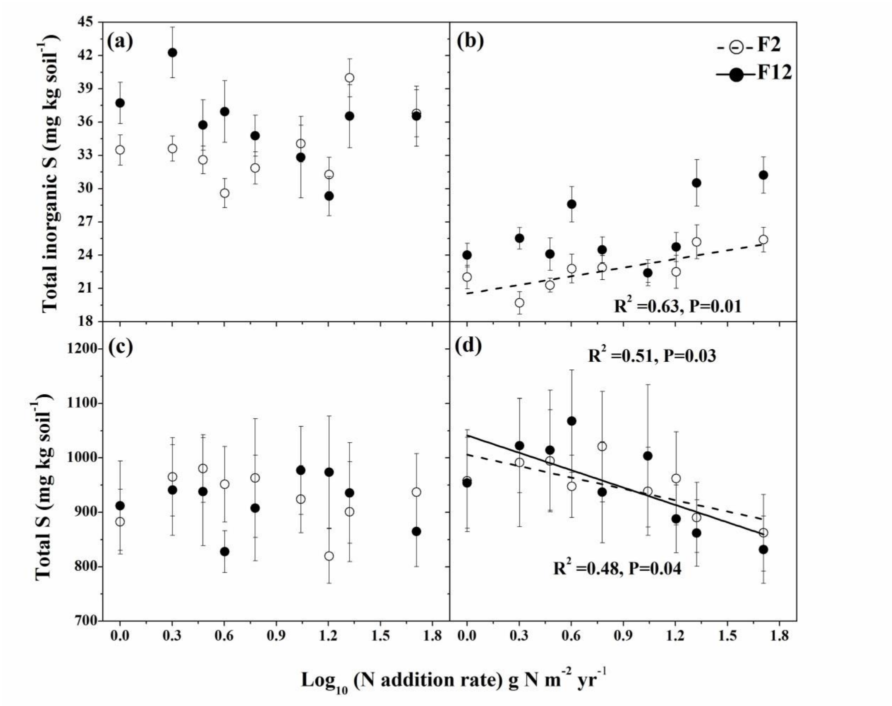

Figure 5 Effects of N addition intensity and frequency on concentrations of soil total inorganic S (a, b) and total S (c, d) in unmown (left) and mown (right) plots. Dashed and solid regression lines correspond to 2 and 12 N additions year^{-1}, respectively. Error bars indicate standard errors.

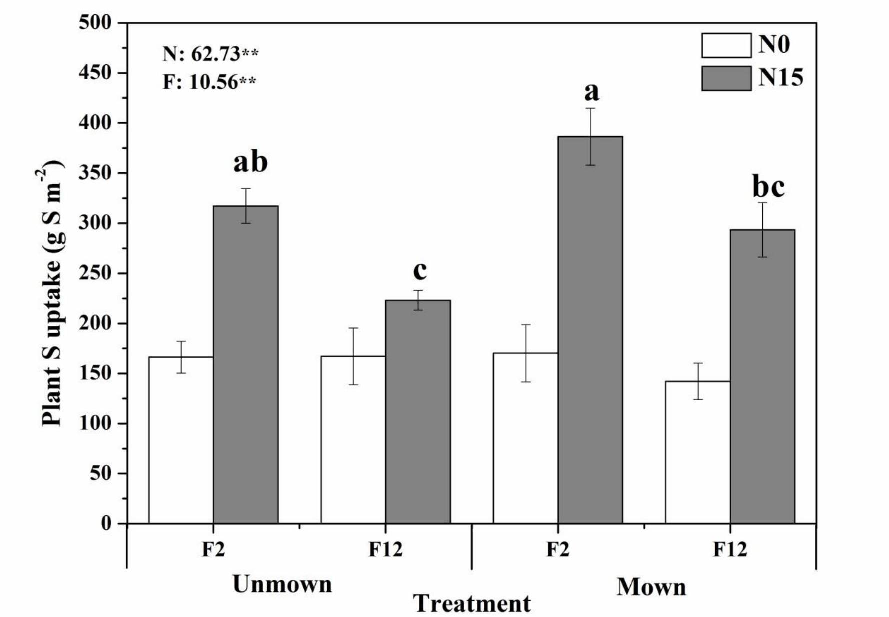

Figure 6 Effect of N addition intensity and frequency on plant S uptake by dominant species of *Leymus chinensis* and *Stipa grandis* without and with mowing (only plant samples in 0 and 15 g N m^{-2} $year^{-1}$ were measured). F2 and F12 indicate low and high frequency of N addition, respectively. Error bars indicate standard error. * and ** represent significance levels (P < 0.05 and 0.01, respectively). Different letters above the bars represent significant difference among means for frequency of N addition and mowing treatments within 0 or 15 g N m^{-2} $year^{-1}$.

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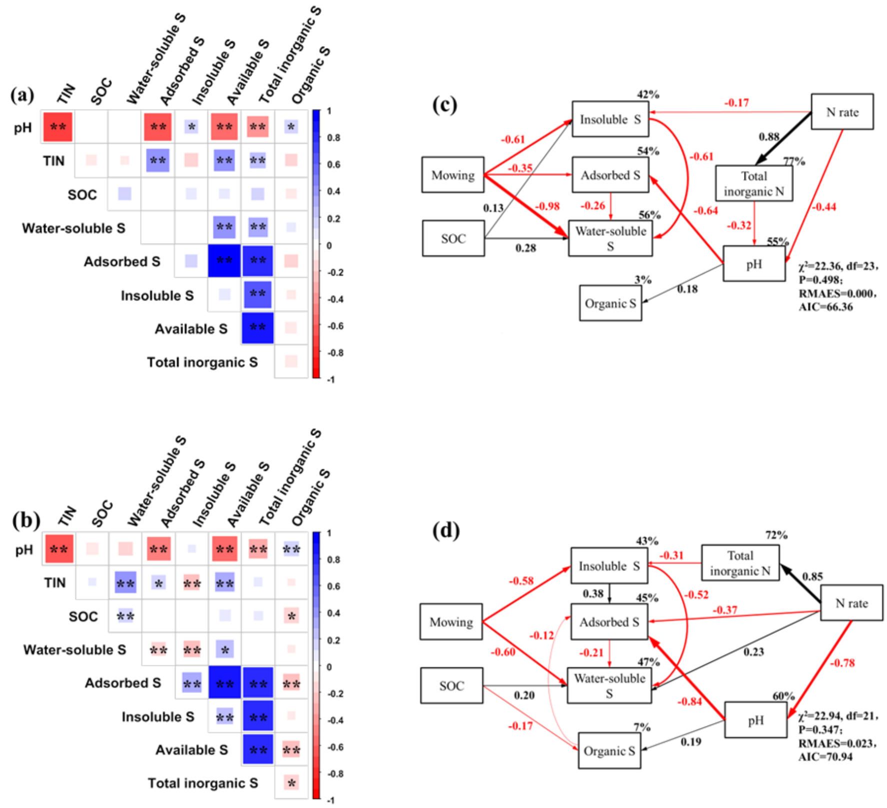

Figure 7 Correlations between soil parameters and inorganic S fractions with low (a) and high (b) frequency of N addition across N addition intensity and mowing treatments; and structural equation modelling (SEM) illustrating the pathways of effects of N addition intensity and mowing on soil parameters and inorganic S fractions under low (c) and high (d) frequency of N addition. For the correlation, * and ** represent significance levels of $P < 0.05$ and 0.01, respectively. For the SEM, 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 final SEM fitted the data well as suggested by the fitting parameters in the figure.