

Effects of nitrogen and water addition on trace element stoichiometry in five grassland species

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Abstract A 9-year manipulative experiment with nitrogen (N) and water addition, simulating increasing N deposition and changing precipitation regime, was conducted to investigate the bioavailability of trace elements, iron (Fe), manganese (Mn), copper (Cu), and zinc (Zn) in soil, and their uptake by plants under the two environmental change factors in a semi-arid grassland of Inner Mongolia. We measured concentrations of trace elements in soil and in foliage of five common herbaceous species including 3 forbs and 2 grasses. In addition, bioaccumulation factors (BAF, the ratio of the chemical concentration in the organism and the chemical concentration in the growth substrate) and foliar Fe:Mn ratio in each plant was calculated. Our results showed that soil available Fe, Mn and Cu concentrations increased under N addition and were negatively correlated with both soil pH and cation exchange capacity. Water addition partly counteracted the positive effects of N addition on available trace element concentrations in

the soil. Foliar Mn, Cu and Zn concentrations increased but Fe concentration decreased with N addition, resulting in foliar elemental imbalances among Fe and other selected trace elements. Water addition alleviated the effect of N addition. Forbs are more likely to suffer from Mn toxicity and Fe deficiency than grass species, indicating more sensitivity to changing elemental bioavailability in soil. Our results suggested that soil acidification due to N deposition may accelerate trace element cycling and lead to elemental imbalance in soil–plant systems of semi-arid grasslands and these impacts of N deposition on semi-arid grasslands were affected by water addition. These findings indicate an important role for soil trace elements in maintaining ecosystem functions associated with atmospheric N deposition and changing precipitation regimes in the future.

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Introduction

Trace elements are essential for plant growth, maintenance, and reproduction in terrestrial ecosystems (Hansch and Mendel 2009). There is increasing evidence indicating that plant productivity and carbon sequestration in terrestrial ecosystems are often limited by element deficiency or toxicity (Alloway 2004; Yadav 2010). For instance, manganese (Mn) toxicity is common in some highly leached tropical soils and can constrain plant growth (Lynch and St Clair 2004). High levels of zinc (Zn) in plants may inhibit metabolic functions and result in retarded growth of both roots and shoots (Fontes and Cox 1998). Iron (Fe) deficiency chlorosis has been reported for soybean grown in calcareous soils in grasslands of the American Midwest (Rogovska et al. 2007). A better understanding of the trace element cycling can help us facilitate the maintenance of community structure and ecosystem functions in terrestrial biosphere under global change (Stevenson and Cole 1999; Ågren 2008).

Nutrient dynamics are generally coupled through biological demand (Sterner and Elser 2002; Knecht and Göransson 2004; Schlesinger et al. 2011), and they vary substantially among environments. Global element imbalances associated with global climate change can affect the elemental composition of plants (Loladze 2002). Two main reasons have been proposed to explain altered plant nutrient stoichiometry due to climate change. First, changing environments alter the internal element demands of plants. For example, the demand of plants for P increases more than the demand for N with increasing atmospheric carbon dioxide (Niinemets et al. 1999). Second, synergistic or antagonistic relationships among elements during their uptake by plants may also alter element stoichiometry in plant tissues (Koukoulakis et al. 2013). It is well known that element concentrations in plants are closely associated with the concentration of elements in soils and their availability for plants (Kabata-Pendias and Mukherjee 2007). Interactions among elements during plant uptake processes can influence the elemental ratios balance within the plant. For example, plants can enhance the uptake of other nutrients under Fe deficiency, suggesting compensatory absorption mechanisms (XianKai et al. 2009). Previous studies have shown that an antagonism exists between Fe and Mn uptake (Somers and Shive 1942), and that an increase in plant Mn content can lead to a decrease of plant Fe content and vice versa. An imbalance of plant element uptake may occur due to different responses of elements

to environmental changes, such as increasing atmospheric nitrogen deposition and changing precipitation regime.

Plant growth is often co-limited by mineral nutrient and water availability in semi-arid grasslands of Northern China (Xu et al. 2012). These ecosystems are sensitive to elevated N deposition and altered precipitation regimes (Niu et al. 2008). Atmospheric N deposition in these regions has significantly increased since the 1980s and are expected to continue to increase over the next 50 years (Liu et al. 2011). In addition, precipitation regimes are expected to change in these ecosystems (Lu et al. 2014). The effect of increased N deposition and altered precipitation regimes on plant growth, biodiversity, ecosystem carbon exchange and N transformation processes have been studied extensively (Xu et al. 2012, 2014). However, the effects of elevated N deposition and altered precipitation on trace element cycling in these regions have not been studied extensively. In terrestrial ecosystems, elevated N and precipitation inputs can increase plant productivity, and hence stimulate consumption of soil trace elements (Vitousek et al. 2010). Nitrogen and precipitation enrichment can also affect the mineralization rate of soil organic matter and alter soil element availability (Harmsen and Vlek 1985). It has been documented that chronic N enrichment tends to induce soil acidification (Bolan et al. 1991) and increase the solubility of trace elements (Wang et al. 2000), leading to potential toxicity (Rashid and Ryan 2004). Bowman and Cleveland (2008) reported that soil acidification and related increases in sol soluble Mn²⁺ and Fe³⁺ were due to N deposition. Increased soil water availability can affect element mobilization and movement in the soil, which will determine the trace element availability for plants (Harpole et al. 2007). Moreover, N and precipitation may interact to affect soil chemistry and ecosystem function (Ochoa-Hueso et al. 2014). Soil water availability can mediate the effect of other environmental factors, including N deposition (Weltzin et al. 2003). For example, water addition can alleviate soil acidification induced by N deposition (Wang et al. 2015). Thus, the impacts of concurrent atmospheric N deposition and precipitation changes on trace element cycling may not be predictable from the effects of N and water, especially in regions limited by both.

A field experiment with N and water addition has been conducted in a semi-arid grassland in northern China since 2005. Soil trace element (Fe, Mn, Cu, and Zn) activation and foliar trace element concentrations in five common plant species were examined to investigate how N and water addition influence the translocation of trace elements in soil–plant system in this grassland. We hypothesized that N addition would (i) enhance trace element solubility in soil via acidification and consequently lead to foliar enrichment of trace elements, (ii) induce an imbalance of trace element composition in soil and plant species, and (iii)

water addition will reduce the effect of N on trace elements in soil–plant system.

Materials and methods

Experimental site and experiment design

A field experiment was conducted in a typical steppe ecosystem dominated by *Stipa krylovii*, *Agropyron cristatum*, *Artemisia frigida* (Xu et al. 2012) in the Restoration Ecological Research Station (116°17'E, 42°02'N, elevation 1324 m a.s.l.) of the Institute of Botany, Chinese Academy of Sciences (IBCAS), in Duolun county, Inner Mongolia, northern China. The site has a chestnut soil (Chinese classification) or Calcisoric Aridisols (the U.S. Soil Taxonomy classification) with a loamy-sand texture (Wang et al. 2014). The mean annual precipitation of the area is around 370 mm with 95% falling in growing season (April to October).

Seven 107 m × 68 m blocks were set up in this steppe in 2005. In a split-plot design, each block was divided into two main plots with water treatment (ambient precipitation and water addition). Different N levels were assigned randomly to the subplots to investigate the influences of N, water and their interactions on trace element dynamics in soil–plant systems. The levels of N addition were 0, 5, 10, 15 g N m⁻² yr⁻¹ in the form of urea, half of which was applied in early May and the other half was applied in late June from 2005 to 2013. From June to August, the water addition plots received 15 mm water weekly via sprinkling irrigation, resulting in a total of 180 mm simulated precipitation each year. Each plot was 8 m × 8 m and replicated seven times, with a 1 m buffer zone between plots. Here, we report on four treatments: control (C), addition of 10 g N m⁻² yr⁻¹ (N), water addition (W) and combined N and water addition (NW).

Sampling and chemical analysis

In August 2013, more than 30 fully-expanded green leaves of each of the five common plant species were randomly collected within each plot. The plant species included two grass species (*Agropyron cristatum* and *Stipa krylovii*), and three forbs (*Artemisia frigida*, *Potentilla tanacetifolia* and *Potentilla bifurca*). In each plot, topsoil samples (0–10 cm) were taken from five random soil cores after removing litter and mixed to form one composite sample.

The leaf samples were dried at 105 °C for 30 min in a drying oven to minimize respiration and decomposition. The samples were washed with deionized water (Jones Jr et al. 1990), and dried at 65 °C to a constant weight. Dried samples were ball-milled, and 150,200 mg samples were

acid digested with a 1:2 mixture of concentrated nitric acid and perchloric acid. Microwave digestion was performed until sample was dissolved into solution. The concentrations of trace elements (Fe, Mn, Cu, and Zn) were analyzed by inductively coupled plasma-Mass Spectrometry (ICP-MS; Perkin Elmer, OPTIMA 3000 DV).

Soil samples were air dried and sieved to 2 mm for the analysis of available trace elements, soil pH, and cation exchange capacity (CEC). Part of each soil sample was ground to pass through a 100-mesh sieve for the analysis of soil total Cu, Zn, Fe, and Mn concentrations. Soil pH was determined in a 1:2.5 soil-to-water suspension with a pH electrode (S210 SevenCompact™, Mettler, Gießen, Germany).

Soil exchangeable calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) were measured by extracting 2.5 g soil (<2 mm) with 50 mL 1 M NH₄OAc (pH=7), and measured by atomic absorption spectrophotometer (AAS, Shimadzu, Japan) at the wavelengths 422.7, 285.2, 766.5, and 589.0 nm. Cation exchange capacity (CEC) was calculated as the sum of charge equivalents of exchangeable cations (Ca²⁺, Mg²⁺, K⁺, and Na⁺), because the soil is calcareous and the sum of exchangeable cations account for more than 99% of the CEC (Aprile and Lorandi 2012; Zhang et al. 2013). Soil diethylenetriamine pentacetic Acid (DTPA)-extractable Fe, Mn, Cu, and Zn were obtained by extracting 10 g soil (<2 mm) with a 20 mL mixture of 0.005 M DTPA + 0.01 M CaCl₂ + 0.1 M triethanolamine (TEA) at pH 7.3 (Lindsay and Norvell 1978), shaking at 25 °C for 2 h filtered through Whatman no. 2v filter paper, and the concentrations were analyzed with AAS.

Soil total Fe, Mn, Cu, and Zn were analyzed by the mixed acid digestion method. Approximately 100 mg of ground soil was weighed and then transferred into a Teflon digestion crucible, and digested with 8 mL HNO₃ + 4 mL HClO₄ + 1 mL HF on a heater plate. The digested solution was decanted into a 25 mL volumetric flask, and diluted with deionized water to constant volume. The concentrations of Fe, Mn, Cu, and Zn were measured using ICP-MS.

To determine the net result of competing processes of elements uptake into plants and elimination from plants including leaf loss and growth dilution (Arnot and Gobas 2006), the bioaccumulation factor (BAF) was calculated as the ratio of the element concentration in leaf to the DTPA-extractable element content in soil (Wang et al. 2013).

Data processing and statistical analysis

Individual soil and plant sample data were averaged and mean values were used for each treatment. The effects of N, water, and their interactions on soil total and DTPA-extractable trace elements (Fe, Mn, Cu, and Zn), foliar trace elements in each plant species, bioaccumulation factors and

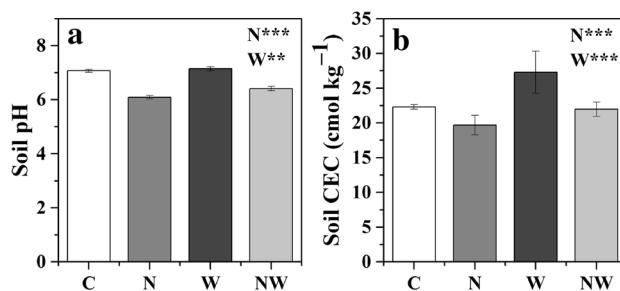


Fig. 1 Responses of soil pH and CEC to nitrogen and water addition. C (control), N (nitrogen addition), W (water addition), and NW (nitrogen and water combination). Values are means of five replicates (\pm SE). Single asterisk, double asterisk, and triple asterisk indicate the significance at $P < 0.05$, 0.01, and 0.001 levels, respectively

foliar Fe:Mn were evaluated by two-way ANOVAs with a split-plot design. Three-way ANOVAs were also performed to test the effects of N, water, species, and their interactions on foliar Fe, Mn, Cu, and Zn contents. Correlations between soil DTPA-trace elements concentrations and soil parameters (pH, CEC, and SOM) were performed using Pearson's Correlation Coefficient. All analyses were performed with SPSS16.0 (SPSS Inc., Chicago, USA).

Results

Properties changes and trace elements in soil

Soil pH significantly decreased under N addition (Fig. 1a, $P < 0.001$), and increased under water addition (Fig. 1a, $P < 0.01$). Soil CEC also decreased with N addition (Fig. 1b, $P < 0.001$), and increased with water addition (Fig. 1b, $P < 0.01$). No interactive effects of N and water were detected on either soil pH (Fig. 1a, $P > 0.05$) or CEC (Fig. 1b, $P > 0.05$). Soil total Cu, Zn, Fe or Mn concentrations did not change among the treatments except for a significant decrease of total soil Mn under N addition and Zn under water addition, compared to the control plots (Table 1; $P < 0.01$ for both).

Nitrogen addition alone significantly increased DTPA—Fe, Mn and Cu concentrations by 245.7, 262.5, and 46.2% respectively (Fig. 2; all $P < 0.001$), but decreased DTPA-Zn concentration by 53.4% (Fig. 2; $P < 0.001$). Water addition significantly reduced DTPA—Fe and Mn concentrations in the soil by 15.2 and 11.4% (Fig. 2; $P < 0.001$, $P < 0.05$, respectively). In addition, N and water addition interacted to affect soil DTPA—Fe and Cu concentrations (Fig. 2; $P < 0.01$, $P < 0.05$, respectively). Nitrogen addition

Table 1 Soil total trace element contents after nine-year nitrogen and water addition

Treatment	Soil total trace element contents ($\mu\text{g g}^{-1}$)			
	Fe($\times 10^3$)	Mn	Cu	Zn
C	22,445.5 \pm 438.0 ^a	483.49 \pm 8.9 ^a	19.44 \pm 2.2 ^a	70.93 \pm 3.4 ^a
N	20,984.2 \pm 579.4 ^a	414.33 \pm 15.1 ^b	18.34 \pm 0.9 ^a	56.83 \pm 2.3 ^{ab}
W	21,423.5 \pm 299.5 ^a	474.79 \pm 16.2 ^a	14.27 \pm 0.7 ^a	55.87 \pm 3.3 ^b
NW	22,269.8 \pm 938.7 ^a	480.10 \pm 17.4 ^a	16.11 \pm 1.6 ^a	58.55 \pm 7.3 ^{ab}

Values are means of five replicates \pm SE

Different letters within each column indicate significant difference at $P < 0.05$ level according to Duncan's Multiple Range Test

C control, N nitrogen addition, W water addition, NW the combination of nitrogen and water addition

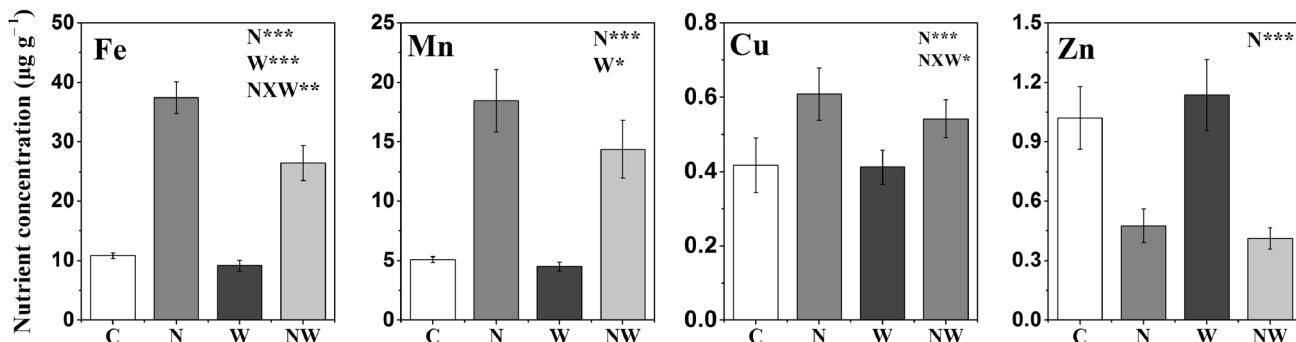


Fig. 2 Responses of DTPA-extractable Fe, Mn, Cu and Zn concentrations in soil to nitrogen and water addition. C (control), N (nitrogen addition), W (water addition), and NW (nitrogen and water combina-

tion). Values are means of five replicates (\pm SE). Single asterisk, double asterisk, and triple asterisk indicate the significance at $P < 0.05$, 0.01, and 0.001 levels, respectively

Table 2 Pearson's correlation coefficients of micronutrients extracted by diethylenetriamine pentaacetic acid (DTPA) with related soil chemical properties ($df=19$)

	DTPA-Fe	DTPA-Mn	DTPA-Cu	DTPA-Zn
pH	-0.96**	-0.96**	-0.80**	0.78**
CEC	-0.49*	-0.48*	-0.35	0.30

CEC cation exchange capacity

* $P<0.05$, ** $P<0.01$ (2-tailed)**Table 3** Results (F values) of the three-way ANOVAs with a split-plot design on the effects of water addition (W), nitrogen addition (N), species (Sp) and their interactions on Fe, Mn, Cu, and Zn concentrations in plant leaves

	Fe	Mn	Cu	Zn
W	4.85	17.21**	1.44	1.27
N	11.10**	116.31***	21.62***	37.10***
Sp	13.95***	27.77***	33.84***	21.76***
W×N	5.46*	22.32***	3.59	3.91
W×Sp	0.77	6.90***	0.90	0.35
W×N×Sp	0.58	8.78***	0.54	1.39

* $P<0.05$, ** $P<0.01$, and *** $P<0.001$

increased DTPA—Fe by 246% under ambient precipitation, but stimulated it by 187.7% under elevated precipitation. Increased precipitation suppressed DTPA—Fe by 15.2% without N addition, but reduced it by 29.2% with N addition. Neither water addition nor N×water interaction influenced soil DTPA—Zn concentration.

Correlations between soil available trace elements and soil properties (Table 2) showed that extractable Fe, Mn, and Cu concentrations were negatively correlated with soil pH and CEC, whereas extractable Zn had positive relationships with soil pH and CEC (Table 2).

Trace elements in plants

When data for all the plant species were analyzed together with 3-way ANOVAs, N addition significantly affected foliar Fe, Mn, Cu, and Zn contents, whereas water addition only affected leaf Mn content (Table 3). All the trace element concentrations varied significantly among species (all $P<0.001$). W×N, Sp and Sp×N interactions were all significant in their effects on leaf Mn concentration.

The results of 2-way ANOVAs on each plant species showed that N addition significantly increased Mn, Cu, and Zn concentrations in all 5 plant species. By contrast, N addition decreased foliar Fe concentration for all the species (Fig. 3). A significant negative effect of water addition was found for foliar Fe in *A. cristatum*, *A. frigida*, and *P. bifurca*, foliar Mn in all species except *S. krylovii*, and

foliar Cu in *P. bifurca* (Fig. 3). Significant interactions between N and water were found for foliar Fe concentration in *A. frigida*, *P. tanacetifolia*, and *P. bifurca* (Fig. 3; all $P<0.05$).

Nitrogen addition significantly raised the foliar Mn concentration in *A. cristatum*, *A. frigida*, *S. krylovii*, *P. tanacetifolia*, and *P. bifurca* by 209, 472, 163, 122, and 353%, respectively. In contrast, water addition decreased foliar Mn concentration in *A. cristatum*, *A. frigida*, *P. tanacetifolia* and *P. bifurca*. The N×W interaction was significant for foliar Cu concentration of *A. cristatum*, *A. frigida*, *S. krylovii* and *P. bifurca*. For foliar Cu, the negative effect of water addition was only significant in *P. bifurca*, and the N×W interaction was significant for *P. tanacetifolia*. The Zn concentration in plants increased with N addition for all species, but there was no water or N×W interaction for foliar Zn concentration.

Nitrogen addition significantly decreased foliar Fe:Mn ratio for the five species (Fig. 4a, d, $P<0.01$; Fig. 4b, c, e, $P<0.001$). After 9 years of N addition, the ratios of Fe:Mn in *S. krylovii*, *A. cristatum* and *P. tanacetifolia* declined to 1.25, 0.45 and 0.33, respectively. No effect of water on the foliar Fe:Mn ratio was detected (Fig. 4; all $P>0.05$). The N×W interaction was significant for foliar Fe:Mn ratio in *A. cristatum* (Fig. 4a, $P<0.01$), *A. frigida* (Fig. 4b, $P<0.001$) and *P. bifurca* (Fig. 4e, $P<0.05$).

Trace element accumulation from soil to plants

N and water addition significantly affected the bioaccumulation factors (BAF) of the four trace elements (Fig. 5). The BAF of Fe decreased significantly under N addition ($P<0.001$ for all the five species). In contrast, N addition increased the BAF of Zn significantly ($P<0.001$ for all five species). The BAF of Mn in *A. cristatum* and *P. tanacetifolia* decreased significantly under N addition ($P<0.01$ for these two species) but BAF of Mn in *S. krylovii* increased ($P<0.01$). Significant negative effect of N on BAF-Cu was only found in *S. krylovii* ($P<0.01$). The BAF of four trace elements did not change significantly in water treatment plots except for a decrease of BAF-Fe (50.4%, $P<0.01$) in *A. frigida*. Moreover, N×W interaction was only significant in BAF-Fe for *A. frigida* ($P<0.01$).

Discussion

Trace element release in soil under nitrogen addition

Consistent with our hypothesis, soil extractable Fe, Mn, and Cu increased with N addition in this semi-arid steppe (Fig. 2). The negative correlations between soil extractable trace element concentrations (Fe, Mn, and Cu) and

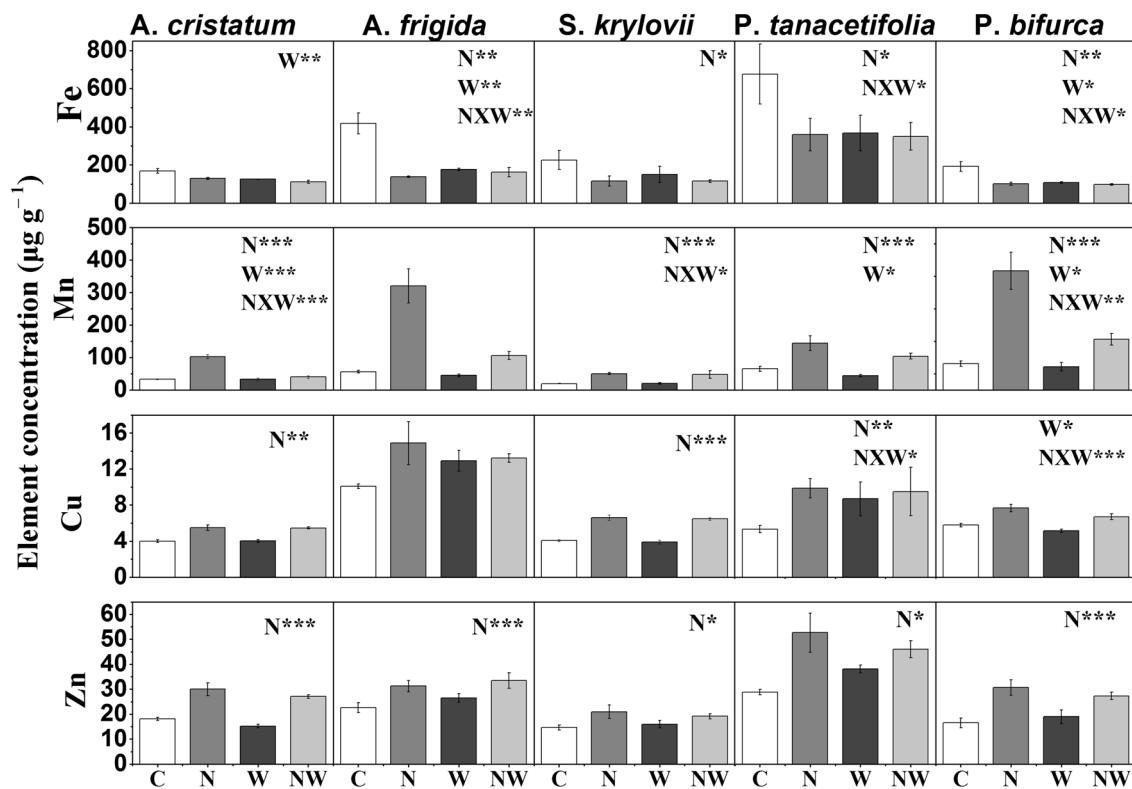


Fig. 3 Responses of leaf Fe, Mn, Cu and Zn concentration to nitrogen and water addition in the five plant species (*Agropyron cristatum*; *Artemisia frigida*; *Stipa krylovii*, *Potentilla tanacetifolia*, and *Potentilla bifurca*) of a semi-arid grassland. C (control), N (nitrogen addition), W (water addition), and NW (nitrogen and water combination).

Values are means of five replicates (\pm SE). Single asterisk, double asterisk, and triple asterisk indicate the significance at $P < 0.05$, 0.01 , and 0.001 levels, respectively

soil pH and CEC indicate that soil trace element activation is associated with soil acidification under N addition. The reduced soil pH and CEC under N addition observed in the present study may be attributed to nitrification of urea and the leaching of exchangeable base cations (Bolan et al. 2003; Musa et al. 2010). Numerous previous studies have reported that concentrations of trace elements increase significantly with decreasing soil pH (Haynes and Swift 1985). This is due to increases in rock weathering and the release of trace elements into soil solution under soil acidification (Bowman and Cleveland 2008). Soil acidification increases weathering and the release of trace elements into soil. Increased H^+ in soil solution induces partial dissolution of carbonates, oxides, and hydroxides bonded to trace element cations, resulting in a higher supply of these ions in available forms.

Our results show decreased amount of soil available Zn in contrast to increased available Fe, Mn and Cu under N addition. It has been widely reported that most zinc in soils occurs in the residual, carbonate, and crystalline iron oxide fractions (Hazra et al. 1987). For example, 60–99 and 5–30% of zinc in soil in China are in the residual fraction and the crystalline iron oxide fraction, respectively (Xiang

et al. 1995). Soil in this grassland may be deficient in Zn, as in many other ecosystems (Iyengar et al. 1981; Wang et al. 2003). The initial release of soil Zn induces enhanced plant uptake, leaving less available Zn remaining in the soil. Previous studies have illustrated that N fertilization can increase (Alloway 2013) or decrease (Malhi et al. 1998) soil available Zn, depending on the relationship between soil supply intensity and plant demand. In this ecosystem, available Zn supply may not meet the enhanced plant uptake given the stimulation of both NPP (Xu et al. 2012) and foliar Zn concentration under N addition.

Imbalanced elemental uptake by plants induced by N deposition

Consistent with our second hypothesis, increased trace element availability in soil enhanced Mn, Cu, and Zn (but not Fe) uptake by plants. To further demonstrate multiple element dynamics in soil–plant system, we evaluated the plant uptake of Fe, Mn, Cu, and Zn from soil using bioaccumulation factor (BAF), the ratio of the chemical concentration in the organism and the chemical concentration in the growth substrate (Reichman 2002; Wang et al. 2013). Values of

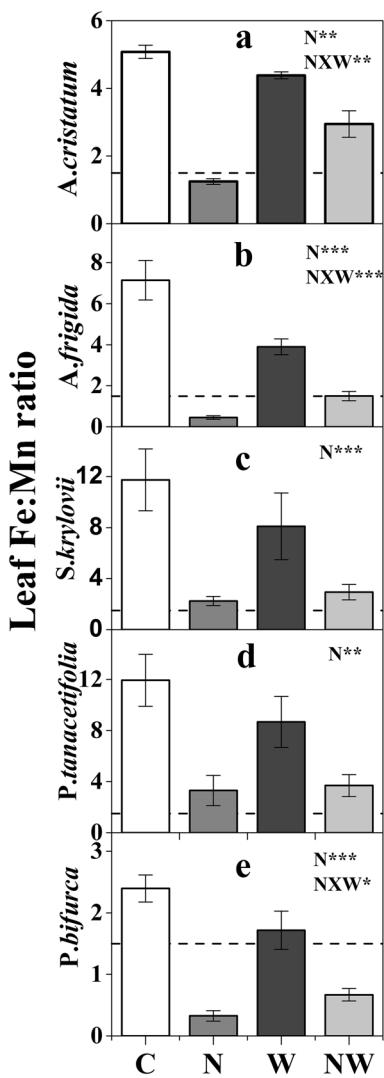


Fig. 4 The ratios of Fe to Mn in the five plant species under nitrogen and water addition. The dotted lines in figure marked at Y value of 1.5, which represent a foliar Fe/Mn critical value for Fe deficiency or Mn toxicity. C (control), N (nitrogen addition), W (water addition), and NW (nitrogen and water combination). Values are means of five replicates (\pm SE). Single asterisk, double asterisk, and triple asterisk indicate the significance at $P < 0.05$, 0.01, and 0.001 levels, respectively

this index differed greatly for trace elements, especially Fe and Zn (Fig. 5). Nitrogen addition could cause Fe efflux from plants to soil and Zn accumulation from soil to plants. The results indicate that different responses of trace elements to long-term N deposition could lead to an imbalance of trace elements in plant tissues.

Since plants acquire most nutrients directly from soil (Han et al. 2011), the increase or decrease of trace elements bioavailability in soil will cause higher or lower concentrations in the plants (Lynch and St Clair 2004). However, the results demonstrated a contrasting response of Fe, with a

decreased Fe absorption despite an increased Fe bioavailability in soil. A dilution effect by increased grassland productivity might cause a lower concentration of Fe in plant tissues (Marschner and Marschner 2012). Additionally, since soil bioavailable Mn also increase under soil acidification and its antagonistic action with Fe in their uptake by plants (Somers and Shive 1942), more bioavailable Fe in acidified soil does not mean more Fe acquisition into plants.

This antagonistic relationship between Fe and Mn is in part attributed to their competition for the absorption sites, since Mn and Fe share many similar physical and chemical properties like atomic masses, radii, and electron structure (Kabata-Pendias 2010). Additionally, there is an interaction during iron and manganese ions transport by membrane transport proteins. Metal transporters are essential to the acquisition of metals with limited bioavailability in soil and also the distribution in plant tissues for healthy plant growth through membrane transport processes (Hall and Williams 2003). Proteins involved in metal transport in plants have already been partly identified, and are clustered into several transporter families like YSL family, ZIP (zinc-and iron-regulated transporter), Nramp family et al. (Hall and Williams 2003). IRT1 (iron-regulated transporter), the first member of the ZIP gene family is identified as the main transporter taking up iron from soil (Varotto et al. 2002). At the same time, IRT1 is a broad-range metal transporter which also participates in the absorption of Mn, Zn and other non-essential metals (Korshunova et al. 1999). Ferrous iron uptake is also facilitated by DMT-1 (Nramp-2, DCT-1) in a pathway shared with manganese (Conrad and Umbreit 2000). Madejczyk and Ballatori (2012) found that the iron exporter ferroportin (FPN1/SLC40A1) can also mediate cellular export of the essential trace element manganese. Our results suggest the hypothesis that the saturated Mn in soil occupied the Mn/Fe transporter, making less Fe acquisition via membrane transport protein, which should be tested experimentally.

Previous studies have found that the optimum Fe/Mn ratio for plant health is between 1.5 and 2.5 (Hedges 2010; Tanaka and Navasero 1966). Plants may suffer from Mn toxicity when foliar Fe/Mn ratio is below 1.5 (Hedges 2010), while Fe deficiency occurs simultaneously. Our observations of declining leaf Fe/Mn ratios for the selected species (Fig. 4) suggest that soil acidification induced by N deposition may cause Fe deficiency and Mn toxicity in steppe species. We also find it remarkable that leaf Fe/Mn ratios dropped more heavily with N addition in the forbs *A. frigida* (~94%) and *P. bifurca* (~86%) than that in the grass *A. cristatum* (~75%, Fig. 4). Additionally, ANOVA analysis showed that responses of metal uptake to nitrogen addition differed significantly among species (Table 2). To uptake Fe from soil, plants have

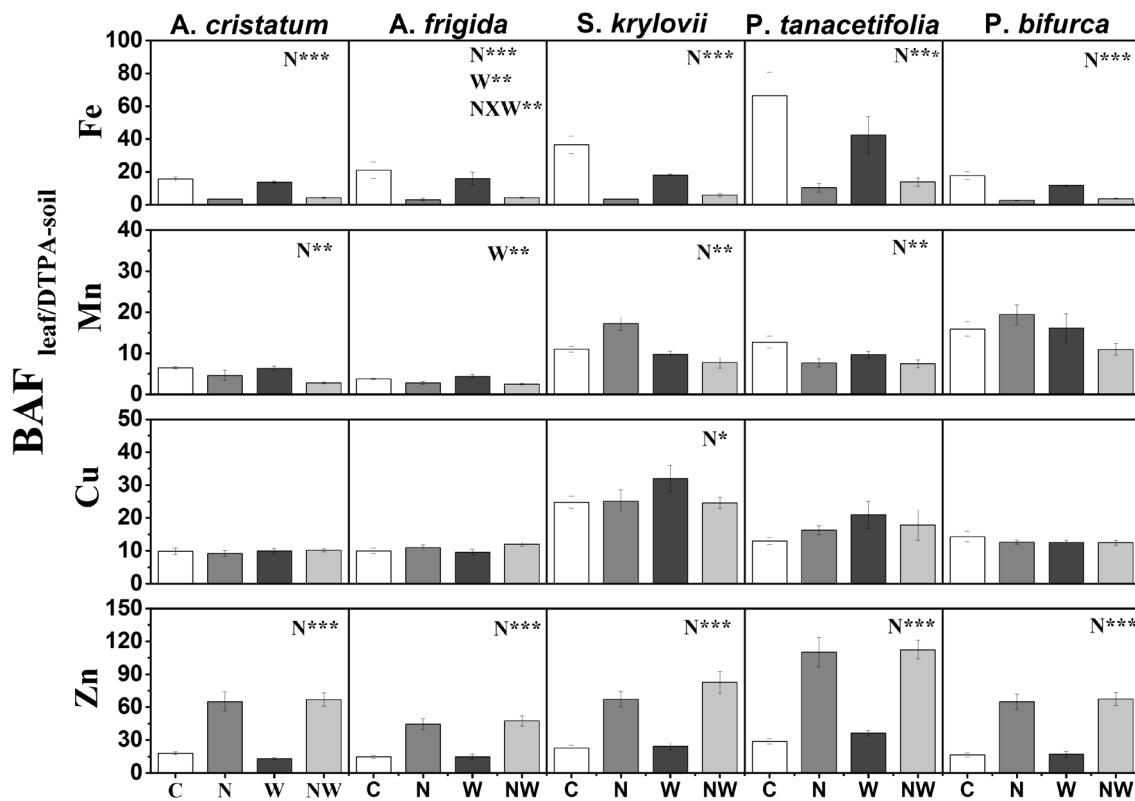


Fig. 5 The variations of BAF_{leaf/DTPA-soil} (micronutrient bioaccumulation factors from soil to the five plant species) under nitrogen and water addition. C (control), N (nitrogen addition), W (water addition), and NW (nitrogen and water combination). The BAF_{leaf/DTPA-soil} here was calculated by the ratio of the element concentration in leaf to the

DTPA-extractable element content in soil. Values are means of five replicates (\pm SE). Single asterisk, double asterisk, and triple asterisk indicate the significance at $P < 0.05$, 0.01 , and 0.001 levels, respectively

developed two well-known high affinity strategies (Strategies I and II). Strategy I plants (forbs included) uptake iron by decreasing the rhizosphere pH thus increasing Fe³⁺ solubility while reducing Fe³⁺ to Fe²⁺ with Fe³⁺-chelate reductases and then plasmalemma transport of Fe²⁺ with transporters. Unlike strategy I plants with the iron acquisition pathway shared with other metals, strategy II plants, (grasses) can release phytosiderophores (PSs) that specifically chelate Fe³⁺ in the rhizosphere. This mechanism can decrease the competitive strength of excessively released metals for uptake sites, thus decreasing the likelihood of Mn toxicity and Fe deficiency. This can result in an ecological advantage for grasses over forbs with the pH-tolerant metal homeostasis. It was recently shown that *A. frigida* is more sensitive to soil acidification than *S. krylovii*, and this may play an important role in the ongoing change in plant community composition from grass-forb codominance to exclusive dominance by grasses in N deposition area (Tian et al. 2015). Our results are consistent with this proposed mechanism for the species loss in ecosystems that are being acidified by N deposition.

Effects of increased precipitation on trace element cycling in soil-plant system

Our study also focused on the effects of increasing water availability in ecosystem trace element behaviors. Similar to previous studies at this site (Wang et al. 2015), increased soil water availability mitigated soil acidification induced by N addition. As a result, mineral solution rates are decreased by increased water input, releasing fewer trace elements into the soil solution. Our results support the hypothesis that responses of trace elements to N deposition are highly influenced by precipitation.

Our results show general decrease of foliar trace elements under water addition. This is reasonable given that decreased release of elements results in lower uptake by plants (Weis and Weis 2004), the increasing plant growth under water addition can also dilute element concentrations in plants. Plants suffering from elemental imbalances may be more sensitive to global changes (Lynch and St Clair 2004). Recently, research ascribed the Mn toxicity and Fe deficiency symptoms in grassland ecosystem via a greenhouse pot experiment (Tian et al. 2016) which

is due to abundance of Mn and also the inhibition of Fe induced by excess Mn rather than just the direct Mn toxicity, which emphasizes the importance of metal homeostasis for plant growth. In our results, the weak responses of trace element BAFs to water addition could be due to the balance between metal activation in soil and plant uptake under increasing precipitation. Water addition alone did not affect foliar Fe/Mn ratio but interacted with N addition, suggesting an interaction between these two environmental changes. Increased water availability ameliorated the negative impacts of nitrogen deposition on soil acidification, avoiding trace element imbalances in the plants.

Conclusions

Our results demonstrate that soil acidification induced by nitrogen deposition causes release of metals in the soil and metal imbalances in the aboveground parts of plants. The ecological advantage of grasses to maintain tissue trace element homeostasis in our results further support the novel mechanism for more forb loss over grasses under nitrogen deposition in temperate steppe. Moreover, the interactive effects between nitrogen deposition and elevated precipitation in this semi-arid grassland indicates that trace element cycling in response to nitrogen deposition and its influence on ecosystem functions are also mediated by other resources that limit plant growth and productivity.

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