



## Clarifying the influence of temperature on variances in plant metallic nutrients through minimizing the effect of precipitation



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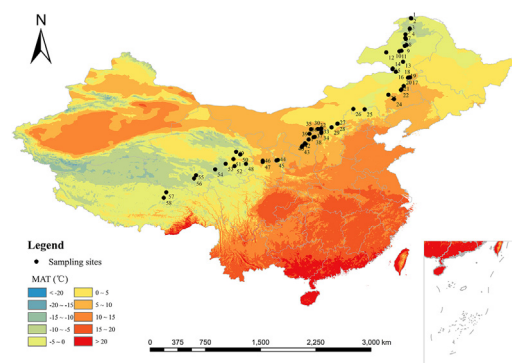
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### HIGHLIGHTS

- Previous work did not separate the effect of temperature from that of precipitation.
- Sampling along 400 mm isohyet was conducted to minimize the effect of precipitation.
- Stable leaf Ca and Mg with temperature was independent of vegetation and soil type.
- Decreased leaf Mn with temperature was not affected by vegetation and soil type.
- Decreased leaf K, Fe and Zn with temperature depended on vegetation and soil type.

### GRAPHICAL ABSTRACT



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### ABSTRACT

Understanding the responses of plant nutrients to climate warming is important in the research of global change. However, the responses of plant metallic nutrients to climate warming have been rarely addressed. Furthermore, in previous field investigations, the influence of temperature on plant metallic nutrients has been not effectively separated from that of precipitation; hence, there exists some uncertainties in the relationships between plant metallic nutrients and temperature. To minimize the effect of precipitation, this study collected plant samples over broad geographical scale along the 400 mm isohyet in China with a temperature span of 14.8 °C. The temperature effects on variations in leaf potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn) and zinc (Zn) were assessed. For all species pooled together, leaf Ca and Mg kept relatively stable, whereas leaf K, Fe, Mn and Zn decreased with increasing temperature. The responses of leaf Ca, Mg and Mn to changing temperature were almost similar at functional group, genus and species levels and independent of vegetation and soil type. It suggested that the relationships between leaf Ca, Mg and Mn and temperature should be general results. However, the patterns of leaf K, Fe and Zn vs. temperature varied across functional groups, genera and species and were affected by vegetation and soil type, which indicated that the observed patterns were local phenomena. Our results suggested that global warming might have no effect on leaf Ca and Mg, but could decrease leaf K, Fe, Mn and Zn.

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## 1. Introduction

Global warming has been observed to exert profound effects on the variances in plant nutrients (Cross et al., 2015; Yue et al., 2017, 2018). Plant nutrient status strongly influences the productivity and function of plant communities (Kerkhoff et al., 2005; Mueller et al., 2010), which have the potential to reflect ecosystem responses to global warming. Thus, investigating the relationship between temperature and variances of plant nutrients is meaningful and necessary in the research of global change.

There have been a large number of studies on this relationship at regional (Han et al., 2005; Kang et al., 2011; Chen et al., 2013; Sardans et al., 2016; Tan and Wang, 2016; Tang et al., 2018) and global scales (Reich and Oleksyn, 2004; Ordoñez et al., 2009; Ma et al., 2018). However, most of the studies focused on the variations in plant nitrogen (N) and phosphorus (P) stoichiometry with temperature; they have neglected the possible influence of temperature change on plant metallic nutrients.

Many metallic nutrients play essential roles in plant physiological functions (Aerts and Chapin III, 2000; Epstein and Bloom, 2004; Marschner, 2012). For example, K is a key activator for the function of >60 enzymes (Smart et al., 1997). Ca maintains the stability of membrane (White and Broadley, 2003) and Mg is an essential component of chlorophyll (Cowan, 2002; Marschner, 2012). Fe and Mn are important for redox system. Zn is involved in membrane integrity and the synthesis of proteins (Broadley et al., 2007).

Several recent papers have addressed the relationships between plant metallic nutrients and temperature over broad geographical scales (Han et al., 2011; Zhang et al., 2012; Sardans et al., 2015; Sun et al., 2015; He et al., 2016). For example, Han et al. (2011) found that leaf K, Ca, Mg and Fe were negatively but leaf Mn was positively correlated with mean annual temperature (MAT) based on data from their field measurements and published literatures. Through analyzing the foliar nutrient concentrations of forest trees across Europe, Sardans et al. (2015) observed that foliar Ca and Mg increased whereas foliar K decreased with increasing MAT. He et al. (2016) collected plant samples from the Alxa Desert and found that leaf Mg and Fe increased with increasing MAT, whereas leaf K, Ca, Mn and Zn did not show significant trends with MAT.

Precipitation can also affect the variations in plant metallic nutrients (Han et al., 2011; Marschner, 2012; Zhao et al., 2016). Thus, it remains unknown to what extent the observed patterns between plant metallic nutrients and temperature are influenced by precipitation. To our knowledge, all previous studies based on field investigations, except Han et al. (2011) in which the influence of precipitation was eliminated using a mathematical method, did not separate the influence of temperature from the effect of precipitation. Hence, it is necessary to further address the relationship between plant metallic nutrients and temperature by minimizing the effect of precipitation across a temperature gradient.

To minimize the effect of precipitation, this study collected plant samples along the 400 mm isohyet extending about 6000 km in China. Our sampling area along this isohyet has a MAT span of 14.8 °C. By measuring the K, Ca, Mg, Fe, Mn and Zn concentrations in plant samples, we expected to reveal the variations in plant metallic nutrients across a temperature gradient when the effect of precipitation was minimized.

## 2. Materials and methods

### 2.1. Site description

The 400 mm isohyet is the dividing line between semi-humid and semi-arid region in China, which extends about 6000 km. A total of 58 sites were set along this isohyet (Fig. 1). The field investigation started from Luoguhecun (site 1, 53°29'N, 122°15'E) in Heilongjiang Province, northeast China to Naqu (site 58, 31°41'N, 91°96'E) in Tibet

Autonomous Region, southwest China. The average of mean annual precipitation (MAP) of these sampling sites is 397.24 mm. Among these sampling sites, Hengshan-1 has the highest MAT of +9.7 °C, while Qumalai has the lowest MAT of −5.1 °C. Detailed information of sampling sites is shown in Table S1.

### 2.2. Plant sampling and chemical analysis

The plant sampling was finished in the summer of 2008 and 2013 respectively. In order to reduce the influence of human activity as much as possible, the sampling sites were chosen 5–7 km from the human habitats. Only healthy and mature leaves were collected during the samplings because the healthy and mature leaves generally have a stable nutrition contents. For tree species, there were a total of eight leaves (two leaves at each of the four cardinal directions) collected from each individual. These sampled leaves were from positions of full-irradiance about 8–10 m above the ground. For herbs and shrubs, the topmost leaves of each individual were collected. There were a total of 658 plant samples collected along the 400 mm isohyet.

All plant samples were cleaned with ultrapure water, oven-dried at 65 °C and ground into fine powders. The K, Ca, Mg, Fe, Mn and Zn concentrations of plant samples were determined by Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) (ICP-OES 7300DV, PerkinElmer, USA) after HNO<sub>3</sub>-H<sub>2</sub>O<sub>2</sub> digestion in a digester (MARS Xpress, CEM, USA). The element concentration of plant samples was expressed as g · kg<sup>-1</sup> for K, Ca and Mg and mg · kg<sup>-1</sup> for Fe, Mn and Zn on a dry mass basis.

### 2.3. Statistical analysis

Leaf K, Ca, Mg, Fe, Mn and Zn concentrations were log<sub>10</sub>-transformed before regression analyses to improve data normality. Bivariate correlation analyses of leaf K, Ca, Mg, Fe, Mn and Zn vs. MAT were performed to obtain the relationships between leaf metallic nutrients and temperature. Partial correlation analyses were conducted to detect the effects of vegetation type and soil type on the correlations between leaf K, Ca, Mg, Fe, Mn and Zn and MAT. Multiple regressions of leaf K, Ca, Mg, Fe, Mn and Zn against MAT, MAP, altitude, vegetation type and soil type were conducted to explore the influences of climate factors, vegetation and soil type on leaf metallic nutrients. One-way ANOVA analysis was used to compare the differences of leaf K, Ca, Mg, Fe, Mn and Zn concentrations across functional groups, genera and species. All statistical analyses were performed using SPSS20.0 (IBM Corporation, USA), with significance being determined at the 0.05 level.

## 3. Results

### 3.1. Variations in leaf metallic nutrients across the temperature gradient for all species pooled together

For all species pooled together, leaf K, Ca, Mg, Fe, Mn and Zn concentrations ranged from 0.24 to 111.87 g · kg<sup>-1</sup>, 0.87 to 109.38 g · kg<sup>-1</sup>, 0.03 to 34.67 g · kg<sup>-1</sup>, 22.4 to 9181.3 mg · kg<sup>-1</sup>, 9.6 to 2476.4 mg · kg<sup>-1</sup> and 1.1 to 7901.3 mg · kg<sup>-1</sup>, with an average of 20.62 g · kg<sup>-1</sup>, 18.82 g · kg<sup>-1</sup>, 5.49 g · kg<sup>-1</sup>, 744.3 mg · kg<sup>-1</sup>, 111.5 mg · kg<sup>-1</sup> and 65.7 mg · kg<sup>-1</sup>, respectively. Leaf K, Fe, Mn and Zn all decreased with increasing MAT ( $r = -0.217, P < 0.001$  for leaf K;  $r = -0.094, P < 0.05$  for leaf Fe;  $r = -0.408, P < 0.001$  for leaf Mn; and  $r = -0.210, P < 0.001$  for leaf Zn), whereas leaf Ca and Mg were not related with MAT (both  $P > 0.05$ ) (Table 1 and Fig. 2).

Partial correlation analyses showed that the relationship between leaf K and MAT was affected by soil type or the combined effect of vegetation and soil type (Table 1). The correlation between leaf Fe and MAT was affected by soil type (Table 1). The relationship between leaf Zn and MAT did not remain when vegetation type, soil type or the combined effect of vegetation and soil type were controlled for (Table 1). The partial

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