Soil properties determine the elevational patterns of base cations and micronutrients in plant-soil system up to the upper limits of trees and shrubs

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Abstract

To understand whether base cations and micronutrients in the plant-soil system change with elevation, we investigated the patterns of base cations and micronutrients in both soils and plant tissues along three elevational gradients and three different climate zones in China. Base cations of Ca, Mg and K and micronutrients of Fe, Mn and Zn were determined in soils, trees and shrubs growing at lower and middle elevations as well as at their upper limits on Balang (subtropical, SW China), Qilian (dry-temperate, NW China) and Changbai (wet-temperate, NE China) mountains. No consistent elevational patterns were found for base cation and micronutrient concentrations in both soils and plant tissues (leaves, roots, shoots and stem sapwood). Rather, soil pH, soil organic carbon (SOC), total soil nitrogen (TN), the SOC to TN ratio (C:N), and soil extractable nitrogen (NO_3^- and NH_4^+) determined the elevational patterns of soil exchangeable Ca and Mg and available Fe, Mn and Zn. However, the controlling role of soil pH and SOC was not universal as revealed by their weak correlations with soil base cations under tree canopies at the wet-temperate mountain and with micronutrients under both tree and shrub canopies at the dry-temperate mountain. In most cases, soil base cation and micronutrient availabilities played fundamental roles in determining the base cation and micronutrient concentrations in plant tissues. An exceptional case existed for the decoupling of leaf K and Fe with their availabilities in the soil. Our results highlight the importance of soil physicochemical properties (mainly SOC, C:N and pH) rather than elevation (i.e., canopy cover and environmental factors, especially temperature), in determining base

cation and micronutrient availabilities in soils and subsequently their concentrations

40   in plant tissues.

Key words base cation, micronutrient, plant tissue, soil physicochemical property,

shrubline, treeline

1 Introduction

Base cations and micronutrients are essential for soil fertility and plant physioecological processes of photosynthesis, metabolism, growth and productivity (Salisbury and Ross, 1992). For instance, exchangeable calcium (Ca) and magnesium (Mg) are predominant base cations responsible in buffering soil acidity, and deficiency of these nutrients can occur in terrestrial ecosystems (Naples and Fisk, 2010; Baribault et al., 2012; Sardans and Peñuelas, 2015), particularly when they are exposed to acid rain. Micronutrient deficiency, on the other hand occurs more frequently, for instance when replenishment of micronutrients via litter decomposition do not keep pace with output processes of plant uptake and leaching (White and Zasoski, 1999; Hernandez-Apaolaza, 2014). High soil pH can limit the availability of micronutrients of iron (Fe), manganese (Mn) and zinc (Zn) (Reisenauer, 1988; Lucena, 2000; Rengel, 2007), while low soil pH can induce toxicities of trace metals constraining terrestrial net primary productivity (He et al., 2005; Reisenauer, 1988; Tian et al., 2016). The concentrations of soil base cations and available micronutrients were suggested to be positively and negatively correlated with soil pH, respectively, but both positively correlated with soil organic matter (SOM) concentration (Reisenauer, 1988; Wang et al., 2017). Quantifying base cation and micronutrient concentrations in soils and plant tissues (leaves, roots, shoots, and stems) can help understand the nutritional status and potential deficiencies of micronutrients during plant growth (Richardson, 2004). However, little attention has been paid to base cation and micronutrient availabilities as well as their inputs and outputs under

changing environmental conditions (Rengel, 2007).

The plant distribution along elevational gradients reflects changes in environmental conditions (Li et al., 2003, 2004, 2008a,b; Zhu et al., 2012a,b). Plants growing at high elevation, especially close to their upper limits, are expected to be

70   highly sensitive to climate change, in particular to global warming (Noble, 1993). Physiological studies of treeline trees have mainly focused on macronutrients (*e.g.*, nitrogen (N), phosphorus (P), and Ca (Richardson, 2004; Liptzin et al., 2013; Mayor et al., 2017), while there are few data available for micronutrients in plant-soil systems along elevational gradients.

75   Two hypotheses have been proposed to explain nutrient accumulation and/or nutrient deficiency in plant tissues at high elevations (Oleksyn et al., 2002; Richardson, 2004). First, the decrease in temperature with increasing elevation declines soil microbial activity and plant metabolism, and thus constrains soil nutrient cycling and plant uptake processes (Körner & Paulsen, 2004; Thébault et al., 2014).

80   At the alpine treeline, low temperature slows down microbial-mediated litter decomposition and thus reduces nutrient supply to plants (van den Driessche, 1974; Richardson, 2004). Second, another paradigm exists that plants retain higher nutrient concentrations in their tissues to maintain metabolic capacity and to avoid cold injury at higher elevations with cold growth conditions (Oleksyn et al., 2002). Whether

85   plants growing at high elevations have higher or lower base cation and micronutrient concentrations is still unclear.

The change in environmental conditions along elevational gradients, including

temperature and growing season length (Barry, 1981), provides a unique opportunity

to examine the spatial distribution of base cations and micronutrients in plant-soil

90   systems. Uncertainties still exists whether soil properties, plant cover, or

environmental factors determine the base cations and micronutrients in plant-soil

systems. We, therefore, studied the base cation (Ca, Mg, and K) and micronutrient (Fe,

Mn, and Zn) concentrations in plant-soil systems along elevational gradients up to the

alpine treeline and shrubline in sub-tropic, dry temperate and wet temperate climate

95   zones in China. Soil base cation and micronutrient concentrations can increase

through soil weathering and decomposition of organic matter, but can decrease with

plant uptake and loss through leaching. At low elevations, forests have closed

canopies and become increasingly more open with elevation. We therefore

hypothesized that soil base cation and micronutrient concentrations increase with

100   increasing elevation because plant uptake decreases more than the supply through

weathering and decomposition with elevation. We also expected that plants at higher

elevations would have greater base cation and micronutrient concentrations in their

tissues (leaves, roots, shoots, and stem sapwood) to maintain physio-ecological

processes in a colder environment. To test these hypotheses, we collected soil and

105   plant samples along three elevational gradients from lower elevations up to the alpine

treeline or shrubline in three different climate zones in China, and studied the Ca, Mg,

K, Fe, Mn, and Zn concentrations in plant-soil systems.

2 Materials and methods

2.1 Site description and sample collection

Study sites were located in three climate zones (summarized in Table S1): Balang

mountain with a subtropical climate located in Wolong Nature Reserve ("subtropical

Mt. ", 102 ̊52′-103 ̊24′E, 30 ̊45′-31 ̊25′N) in southwestern China, Qilian mountain

located in the dry-temperate climate zone ("dry-temperate Mt. ", 102 ̊58′-103 ̊01′E,

37 ̊14′-37 ̊20′N) in northwestern China, and Changbai mountain with a wet-temperate

climate ("wet-temperate Mt.", 126 ̊55′-129 ̊00′E, 41 ̊23′-42′36 ̊N) located in

northeastern China (see Fig. S1). The subtropical Mt. is influenced by warm-wet

monsoon masses in summer and continental air masses in winter (Li et al., 2012). The

mean annual precipitation (MAP) of the subtropical Mt. is about 846 mm monitored

by Dengsheng Meteorological Station at 2730 m (Li et al., 2012). For the

dry-temperate Mt., the MAP is 435 mm, which is monitored by the Qilian weather

station at 2787 m altitude (Qiang et al., 2003). The wet-temperate Mt. is located in a

typical continental temperate monsoon climate zone with MAP increasing from 632 to

1154 mm along the elevational gradient from 530 to 2200 m (Shen et al., 2013).

In this study, the alpine treeline and shrubline are defined as the upper limit of

obvious trees and shrubs, respectively. The trees that were investigated are *Abies*

faxoniana (elevation range 2860-3670 m) for subtropical Mt., *Picea crassifolia*

(elevation range 2540-3250 m) for dry-temperate Mt., and *Betula ermanii* (elevation

range 1700-2030 m) for wet-temperate Mt.. The shrubs are *Quercus aquifolioides*

(elevation range 2840-3590 m), *Salix gilashanica* (elevation range 3020-3540 m), and

Vaccinium uliginosum (elevation range 1430-2380 m) for the subtropical,

dry-temperate and wet-temperate Mts., respectively. The targeted treeline trees and shrubline shrubs are dominant and common species, respectively, for each study site. The soils from the three sampling sites of subtropical, dry-temperate, and wet-temperate were classified as Umbric Cryic Cambisols, Calcaric Ustic Cambisols and Andic Gelic Cambisols, respectively (IUSS Working Group WRB, 2014).

Plant tissue samples of current-year mature leaves, roots (< 2 mm), stem sapwood, and shoots (twigs) from trees and shrubs were collected at lower and middle elevations, as well as at the upper limits. At each elevation, 6 plots (10 m × 10 m) were selected to serve as 6 replicates on southern slopes. Within each plot, 6-10 trees or shrubs of similar height were randomly selected for tissue sampling. Soils (0-10 cm) were directly collected under the canopy of trees or shrubs sampled for each plot using a 3-cm diameter corer. Both plant and soil samples were homogenized and composited within each plot. Samples were collected at the middle of July for subtropical Mt., at the beginning of August for dry-temperate Mt., and at end of August for wet-temperate Mt. in 2014. The main characteristics of the three study sites are summarized in Table S1.

2.2 Chemical analysis

The soil samples were separated into two parts with one part being air-dried to constant weight and the other stored at 4 ℃ for further analyses. For subtropical and wet-temperate Mts., soil organic carbon (SOC) and total nitrogen (TN) was determined on ground soils using an elemental analyzer (Vario MACRO Cube, Elementar, Germany). For dry-temperate Mt., the ground soil samples were treated

with 12 M HCl according to Wang et al. (2015) to remove inorganic C before organic

155 C determination on the elemental analyzer. Soil NO_3^--N and NH_4^+-N were extracted

from fresh soils with a 2 M KCl solution and measured using an AutoAnalyser III

continuous Flow Analyzer (Bran & Luebbe, Norderstedt, Germany). Soil total

inorganic nitrogen (TIN) was the sum up of extractable NO_3^--N and NH_4^+-N. Soil

Olsen phosphorus (P) was quantified by colorimetric analysis after extraction with a

160 0.5 M $NaHCO_3$ solution (Olsen et al., 1954).

A subsample of 5 g soil was used to determine soil pH in a 1:5 (w/v) soil-to-water

extract. Soil exchangeable base cations were extracted with a 1 M ammonium acetate

solution (Wang et al., 2017). Soil available micronutrients were extracted by

diethylenetriamine pentaacetic acid (DTPA) according to Lü et al. (2016). Briefly, 10

165 g of soil was extracted by 20 ml 0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA

(triethanolamine) (pH 7.0). The soil-solution suspension was shaken for 2 h at 180

rpm and then filtered through ash-free filter paper. The concentrations of base cations

and micronutrients were determined using an atomic absorption spectrometer (AAS,

Shimadzu, Japan).

170 Plant samples of leaves, roots, shoots and stem sapwood were oven-dried and

ground for base cation and micronutrient analyses. Root samples were washed prior to

being oven-dried. To determine total base cation and trace element concentrations, 0.2

g plant samples were digested with a mixture of acids of HNO_3 and $HClO_4$ (5:1, v/v)

on a hot plate. After the mixture turned into clear solution, the digests were decanted

175 into a 50 ml volumetric flasks and the volume was adjusted into 50 ml. The

concentrations of Ca, Mg, K, Fe, Mn, and Zn were determined by the AAS (Shimadzu, Japan).

2.3 Statistical analyses

Normality of data was determined using the Kolmogorov-Smirnov test, and homogeneity of variances using Levene's test. Two-way ANOVAs were executed to determine the effects of plant life form (tree or shrub), elevation position and their interactions on soil pH, SOC, soil exchangeable base cations and available micronutrients, and total base cations and micronutrients in plant tissues. We assigned sampling site as a random factor in the statistics, as this study aimed to test the general elevational patterns instead of site-specific heterogeneity of base cations and micronutrients in plant-soil system across three sites. Within each site, the effect of elevation on measured parameters was determined by multiple comparisons with a Tukey design for soils and each life form. Pearson correlation analysis was used to determine the relationships between measured parameters. All statistical analyses were performed in SPSS 16.0 (SPSS, Inc., Chicago, IL, USA) and statistical significance was accepted at $P < 0.05$.

3 Results

3.1 Soil pH and SOC

Soil pH was significantly different among elevational positions (Table 1). For both subtropical and dry-temperate Mts., soil pH decreased with increasing elevation under tree canopy, while it was the opposite trend under shrub canopy (Fig. 1a). For

wet-temperate Mt., the upper limit of shrubs had significantly higher soil pH (Fig. 1a).

For all three sites, SOC concentration showed a hump-shaped trend with the

highest value at the middle elevation under tree canopy (Fig. 1b). Under shrubs, SOC

concentration significantly increased with increasing elevation for subtropical and

wet-temperate Mts., while it was the lowest at the upper limit of shrubs for

dry-temperate Mt. (Fig. 1b).

3.2 Changes in soil base cations and available micronutrients

Soil exchangeable Ca and Mg decreased with increasing elevation under tree canopy

of subtropical and wet-temperate Mts. and under shrubs of dry-temperate Mt. (not for

Mg) (Fig. 2a,b). However, they showed the opposite trend under shrubs of subtropical

(not for Mg) and wet-temperate Mts. and under trees of dry-temperate Mt. (Fig. 2a,b).

Soil exchangeable K decreased with increasing elevation under tree and shrub

canopies at subtropical Mt. and under trees at dry-temperate Mt. (Fig. 2c).

Soil available Fe was significantly affected by elevation position (Table 1, Fig.

2d). The upper limit had the lowest concentration under both tree and shrub canopies

for subtropical Mt. and under shrub canopy for wet-temperate Mt. (Fig. 2d). For

dry-temperate Mt., soil available Fe significantly increased with increasing elevation

under both tree and shrub canopies (Fig. 2a). For subtropical Mt., soil available Mn

was significantly higher at the middle elevation under tree canopies. Soil available Mn

decreased with increasing elevation under shrub and tree canopies for subtropical and

wet-temperate Mts., respectively, while it showed the opposite trend under both tree

220 and shrub canopies of dry-temperate Mt. and under shrubs of wet-temperate Mt. (Fig.

2e). Soil available Zn was significantly affected by plant life form and the interactive

effect between life form and elevation position (Table 1). Specifically, soils at the

upper limit had the highest available Zn under shrubs at wet-temperate Mt..

225 **3.3 Base cations in plants**

For subtropical Mt., a significant decrease of Ca concentration was detected in the

leaves of trees and shrubs (Fig. 3a), roots and shoots of trees (Fig. 3b,c), and stem

sapwood of shrubs (Fig. 3d). For dry-temperate Mt., Ca concentration decreased with

increasing elevation in roots, shoots and stem sapwood of trees (Fig. 3b,c,d), and in

230 shoots and stem sapwood of shrubs (Fig. 3c,d). For wet-temperate Mt., shoot Ca

concentration decreased with increasing elevation for trees (Fig. 3c). Along with

increasing elevation, a significant decrease of Mg was found in shrub leaves, tree

roots, shrub shoots and stem sapwood at sub-tropical Mt. (Fig. 3e,f,g,h), and in roots,

shoots and stem sapwood of trees at dry-temperate Mt. (Fig. 3f,g,h), and in leaves and

235 shoots of both trees and shrubs at wet-temperate Mt. (Fig. 3e,g). With the increase in

elevation, K concentration significantly decreased in leaves of trees, roots and stem

sapwood of both trees and shrubs at subtropical Mt. (Fig. 3i,j,l), in tree shoots of

dry-temperate Mt. (Fig. 3k), and in leaves of both trees and shrubs at wet-temperate

Mt. (Fig. 3i).

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3.4 Micronutrients in plants

For subtropical Mt., Fe concentrations in leaves (Fig. 4a) and roots (Fig. 4b) showed a similar trend with soil available Fe, with the highest values at the middle elevation for both trees and shrubs. For dry-temperate Mt., the highest Fe concentrations were

245 found at the lower elevation in leaves (Fig. 4a), roots (Fig. 4b), shoots (Fig. 4c) and stem sapwood (Fig. 4d) for trees and in shoots (Fig. 4c) and stem sapwood (Fig. 4d) for shrubs. For wet-temperate Mt., Fe concentration was the highest in tree shoots at the middle elevation (Fig. 4c), in shrub leaves at lower elevation (Fig. 4a), and in roots, shoots and stem sapwood of shrubs at the upper limit of trees (Fig. 4b,c,d).

250 The Mn concentration decreased with increasing elevation in leaves and shoots of both trees and shrubs at subtropical Mt. (Fig. 4e,g), in stem sapwood of shrubs at both subtropical and dry-temperate Mts. (Fig. 4h). The Mn concentration increased with increasing elevation in leaves of trees at dry-temperate Mt. (Fig. 4e), in roots of both trees and shrubs at wet-temperate Mt. (Fig. 4f), in shoots of shrubs and stem sapwood

255 of trees and shrubs at wet-temperate Mt. (Fig. 4g,h).

The Zn concentration was the highest at middle elevation for trees in leaves at wet-temperate Mt. (Fig. 4i), in roots at dry-temperate Mt. (Fig. 4j), in shoots at wet-temperate Mt. (Fig. 4k) and in stem sapwood at subtropical Mt. (Fig. 4l). With the increase in elevation, a decrease of Zn concentration was found in roots of trees at

260 subtropical Mt. (Fig. 4j) and in stem sapwood of shrubs at dry-temperate Mt. (Fig. 4l); however, an increase of Zn was found in shrub roots at wet-temperate Mt., in shoots of trees at dry-temperate Mt. and shrubs at wet-temperate Mt. (Fig. 4k), and in stem sapwood of trees at dry-temperate Mt. and shrubs at wet-temperate Mt. (Fig. 4l).

3.5 Correlations

Across all sampling sites and plant life forms, both soil exchangeable Ca and Mg were positively correlated with soil pH (Fig. S2a,b) and TN (Table 2), while they were negatively correlated with soil C:N, NO_3^-, and NH_4^+ (Table 2). For wet-temperate Mt., both soil pH and SOC showed no relationship with soil exchangeable Ca and Mg under tree canopies, although SOC was positively related to exchangeable K (Table S2). Negative correlations were found between stem and leaf for both Mg and K concentrations (p < 0.01; Table S3). However, Mg and K concentrations in roots showed no correlation with that in leaves (Table S3).

When analyzing data across sampling sites and plant life forms, soil available Fe, Mn and Zn negatively correlated with soil pH ($p < 0.01$; Table 2, Fig. S2c,d,e), and soil available Fe and Zn positively correlated with SOC ($p < 0.01$; Table 2). However, available micronutrients had no relationships with both soil pH and SOC at dry-temperate Mt., except for a positive correlation between soil pH and available Mn under shrub canopies (Table S2). For both Mn and Zn concentrations, significant and positive correlations were found between soil and plant tissues (Table S3). However, soil available Fe negatively correlated with Fe concentrations in shoot and stem ($p < 0.01$; Table S3).

4 Discussion

4.1 Elevational patterns of base cations and available micronutrients in soils and

relationships with pH and SOC

Contrary to our first hypothesis, no consistent elevational patterns were detected for soil exchangeable base cations and available micronutrients under either trees or shrubs. Inconsistent elevational patterns of soil base cations and available

290   micronutrients indicated that plant uptake of these nutrients did not necessarily decrease more than nutrient supply at higher elevation due to more open canopies. Our results suggest that soil physiochemical parameters were the dominant contributors and more important than environmental gradients affecting elevational patterns of soil exchangeable base cations and available micronutrients (Fig. S2, Table

295   2). For instance, soil available Fe, Mn and Zn followed patterns of SOC under trees along the elevational gradient at subtropical Mt. (Table S2), while for shrubs at subtropical Mt., soil pH, instead of SOC, regulated elevational patterns of soil available Fe, Mn and Zn (Table S2). Our findings are consistent with a vast amount of previous studies confirming the pivotal role of soil pH and SOC concentration in

300   determining soil base cation and micronutrient availabilities (Sharma et al., 2004; Lü et al., 2016; Wang et al., 2017). However, the fundamental roles of SOC and soil pH in controlling soil base cation and micronutrient availabilities, was not universal as suggested by the relatively weak relationships of soil pH and SOC with soil base cations under tree canopies at wet-temperate Mt. and with micronutrients under both

305   tree and shrub canopies at dry-temperate Mt. (Table S2). This could indicate species-specific responses and effects on soil base cation and micronutrient availabilities.

Other soil parameters, such as C:N and extractable NO_3^- and NH_4^+ also influenced the availability of base cations (Table 2). Soil C:N ratio serves as an indicator of SOM decomposition status where more decomposed SOM possesses a lower C:N ratio (Sollins et al., 2009) and a higher content of negatively charged functional groups (i.e. phenolic, carboxyl, and hydroxyl groups) (Haberhauer et al., 1998). In this study, negative correlations between soil C:N and base cations (Table 2) suggest that more decomposed SOM is beneficial for the retention of soil base cations. Furthermore, soil with a higher level of extractable NO_3^- predisposes cations to leach accompanied by loss of NO_3^- (Cremer and Prietzel, 2017). Therefore, significant negative correlations were detected between soil NO_3^- and base cations in this study (Table 2). Soil extractable NH_4^+ was also negatively correlated to exchange with Ca and Mg, possibly because NH_4^+ can exchange with base cations on surface soil colloids into soil solution thereby enhancing their loss (Wang et al., 2015; Cusack et al., 2016).

A negative correlation between soil pH and soil available micronutrients (Table 2) might be due to precipitation of micronutrient cations at higher soil pH (Rengel, 2007). Indeed, solubility of micronutrients was suggested to decrease from 100-fold (for Mn and Zn) to 1000-fold (for Fe) with one-unit increase of soil pH (Rengel, 2001). Soil organic matter plays an important role in micronutrient retention due to its negative charge (He et al., 2005; Wang et al., 2015, 2017). This may be a reason for the positive relationships between SOC and micronutrients (although not always significant, Table 2). While no general patterns were found for distribution of

330   micronutrients under both tree and shrub canopies with elevation, our results suggest

that the determinants of soil micronutrient availabilities were soil pH and SOC

concentration, which are reflections of long-term climatic conditions, plant-soil

interactions, and biogeochemical processes (Sinsabaugh et al., 2008).

335   **4.2 Elevational patterns of base cations and micronutrients in plants and**

plant-soil system

In contrast to our second hypothesis, both trees and shrubs at higher elevation did not

necessarily contain higher base cation and micronutrient concentrations in their

tissues. No general patterns were found for base cations and micronutrients in both

340   trees and shrubs along elevational gradients across the three sites (Fig. 3,4). Even

normalizing the data to per unit concentration of soil available nutrients, there were

still no consistent elevational patterns for both base cations and micronutrients in

plant tissues (Fig. S3). This suggests that base cation and micronutrient concentrations

in plants are influenced by other factors besides elevation-induced changes in

345   temperature, precipitation, specific nutrient absorption characteristics of different

tissues, soil base cation and micronutrient availabilities and other edaphic properties

(Campo-Alves, 2003; Richardson, 2004). Another explanation could be that initial

differences in soil properties (*e.g.*, parent material) among elevation positions were

larger than the effects of elevation. Soil base cation and micronutrient availabilities

350   were an important factor influencing their concentrations in plant tissues across all

plant species and sampling sites (Table S3). Similar results were found for

macronutrients (i.e. nitrogen and phosphorus) suggesting that "plants are what they root in" (Elser et al., 2010; Han et al., 2014). However, plant nutrients did not covary with soil nutrients along a 2200 km-long climatic gradient in grasslands of northern

355 China (Luo et al., 2015, 2016). The discrepancy of our study with Luo et al. (2015, 2016) might be driven by different ecosystem types (forest vs. grassland), dominant climatic factor gradients (temperature vs. precipitation), and different soil properties. The studies of Luo et al. (2015, 2016) were conducted in grassland ecosystems where precipitation played an essential role in nutrient concentrations in plant-soil systems.

360 Moreover, base cation and micronutrient cycling processes are likely to be different between high-organic and fine-grained forest soils in our study versus low-organic and sandy grassland soils in Luo et al. (2016). Inconsistent elevational patterns in plant nutrient concentrations could also be derived from the fact that individual plant species reinforced patterns of soil nutrient availabilities in their vicinity causing a

365 positive feedback between plant and soil (Hobbie, 1992).

The topic of base cation and micronutrient translocation in intact plant is important as it deals with the movement of micronutrients from root to the leaves for physiological activities, such as photosynthesis (Welch and Shuman, 1995). Also, it is an important process in determining plant chemical composition and subsequently

370 litter quality, litter decomposition and nutrient release (Sun et al., 2016). Given earlier findings that transport of base cations from roots to the leaves in woody plants is slow (van der Heijden et al., 2015), we found no significant correlation for both Mg and K between roots and leaves (Table S3). However, negative relationships of stem Mg vs.

leaf Mg and stem K vs. leaf K suggest that the plant internal pools of base cations

375   could act as sources of base cation supply for leaves (Weatherall et al., 2006).

Translocation of base cations within plant tissues is one of the main physiological

mechanisms buffering low nutrient availabilities in soils (van der Heijden et al., 2015).

For instance, supplementation of Mg is a critical process to maintain photosynthesis in

forests growing on acid and cation poor soils (Verbruggen and Hermans, 2013). In

380   support of this, we found significantly positive correlations between Mg and soluble

sugar concentrations (one of the main photosynthate) in leaves across the three sites

(Fig. S4a), while relationships were more pronounced at wet-temperate Mt. (Fig.S4b)

where soil pH and exchangeable Mg was the lowest (Fig. 1a, 2b).

Unlike Ca, Mg, Mn and Zn, the concentrations of K and Fe in plant leaves

385   decoupled with their availabilities in the soil (Table S3), which may suggest that not

only availability of these nutrients in soils affect their leaf concentrations, but that also

other environmental factors (*e.g.*, temperature) played more important roles in

affecting plant nutrition (van den Driessche, 1974). We do not know why this

decoupling only occurred for K and Fe, but possibly factors such as temperature

390   constrained soil microbial activity and plant metabolism (Körner and Paulsen, 2004)

and subsequently uptake of these nutrients by plants. On the other hand, plants often

increase nutrient uptake to compensate for decreased metabolism at low temperature

(Reich and Oleksyn, 2004). Thus, these opposite effects of temperature on K and Fe

concentrations in plant tissues may have obscured their relationships with K and Fe

395   availability in the soil along the elevational gradients. While plant nutrient

concentrations were mainly influenced by nutrient availabilities in the soil and by plant-internal translocation processes, we found no consistent evidence that plants accumulate more base cations and micronutrients in their tissues to better adapt to cold environments at higher elevation.

400

5 Conclusions

We did not find consistent elevational patterns of base cations and micronutrients in plant-soil systems along three different elevation transects up to the alpine treeline and shrubline in different regions of China. Rather, our results highlight the essential

405 roles of specific edaphic properties of soil pH, SOC and extractable nitrate and ammonium in regulating soil base cation and micronutrient availabilities among sampling sites. Soil available base cations and micronutrients were mostly positively correlated to concentrations of base cations and micronutrients in plant tissues, except for K and Fe. Our results suggest that base cation and micronutrient concentrations in

410 plants located in upper limits of the tree- and shrublines are largely controlled by their availabilities in the soil, rather than by plant adaptations to avoid cold injury at higher elevations.

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Data Availability

Data sets for this paper can be obtained via personal communication.

Author contributions

Mai-He Li designed the study; Xue Wang, Heyong Liu, Jinfei Yin and Zhan Shi did the plant and soil measurements; Ruzhen Wang and Xue Wang analyzed the data; Ruzhen Wang wrote the manuscript; Mai-He Li, Feike A. Dijkstra and Artemi Cerdà revised the manuscript; Mai-He Li and Yong Jiang provided funding and laboratory facilities for this study.

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Tables

 Table 1 Two-way ANOVAs (*F* values) of the effect of plant life form (L, tree or shrub), elevation position (E), and their interactions on soil pH, soil organic carbon (SOC), base cations and micronutrients in soils (exchangeable/available form) and plants (total) across sampling sites.

	soil pH	SOC	soil Ca	Soil Mg	Soil K	leaf Ca	root Ca	shoot Ca	stem Ca	leaf Mg	root Mg	Shoot Mg	stem Mg	leaf K	root K	shoot K	stem K	soil Fe	soil Mn	soil Zn	leaf Fe	root Fe	shoot Fe	stem Fe	leaf Mn	root Mn	shoot Mn	stem Mn	leaf Zn	root Zn	shoot Zn	stem Zn
L	0.18	5.75*	1.02	8.15**	24.64**	9.74**	1.69	10.85**	39.36**	93.41**	8.35**	15.82**	118.58**	12.57**	6.41*	63.56**	87.00**	8.72**	2.60	6.89**	64.67**	0.72	0.12	8.54**	0.03	27.05**	40.67**	25.17**	11.83**	2.01	4.40*	80.60**
E	3.16*	10.12**	1.22	1.71	15.21**	7.82**	3.85*	8.36*	1.17	0.83	5.85**	2.42	10.47**	9.30*	0.22	0.48	0.85	5.75**	1.54	0.01	0.02	1.80	0.50	2.40	7.13**	2.63	1.54	0.53	0.67	0.71	1.67	3.61*
L×E	15.57**	3.34*	2.40	0.55	8.61*	2.24	8.55**	6.61*	1.11	1.82	7.20**	5.23*	0.37	0.96	1.01	2.20	2.84	0.10	1.69	4.42*	0.36	3.40*	3.73*	0.61	0.15	2.81	1.22	2.37	0.90	1.93	1.34	2.12

* and ** indicate significant level at P < 0.05 and 0.01, respectively.

Table 2 Regression statistics relating soil base cations and micronutrients to other soil
physicochemical parameters under all trees and shrubs across three sampling sites.
The TN, C:N, TIN, and Olsen P represent soil total nitrogen, SOC to TN ratio, total
inorganic nitrogen, and Olsen phosphorus.

	Ca	Mg	K	Fe	Mn	Zn
pH	**0.94**[**]	**0.87**[**]	-0.06	**-0.64**[**]	**-0.36**[**]	**-0.73**[**]
SOC	0.12	0.24[*]	0.18	0.30[**]	0.07	**0.37**[**]
TN	**0.39**[**]	**0.51**[**]	0.18	**-0.01**	0.03	0.11
C:N	-0.45[**]	-0.44[**]	-0.11	**0.60**[**]	**0.02**	0.44[**]
NO_3^-	-0.24[*]	**-0.29**[**]	**0.28**[**]	**-0.05**	0.02	0.08
NH_4^+	-0.38[**]	-0.33[**]	-0.10	0.46[**]	0.15	**0.63**[**]
TIN	-0.38[**]	-0.39[**]	0.19[*]	0.17	0.08	0.36[**]
Olsen P	0.10	0.18	**0.49**[**]	-0.05	0.04	0.03

[*] and [**] indicate significant level at $P < 0.05$ and 0.01, respectively.

Figures

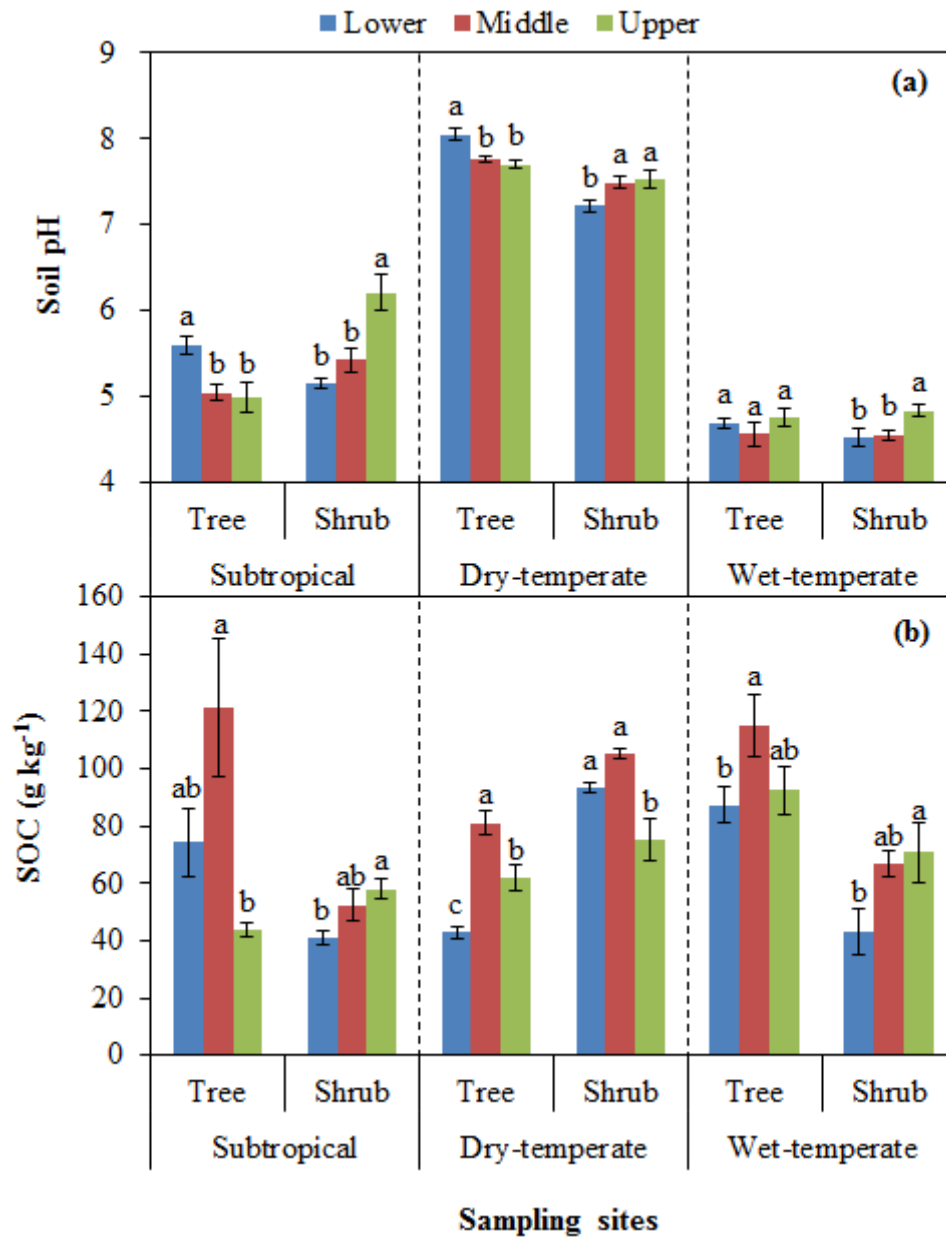



Fig. 1 Soil pH values (a) and soil organic carbon (SOC) concentration (b) at lower

and middle elevations as well as at the upper limit of treelines or shrublines for each

 of the three sites. Different letters indicate significant differences among three

elevations within treeline or shrubline for each site.

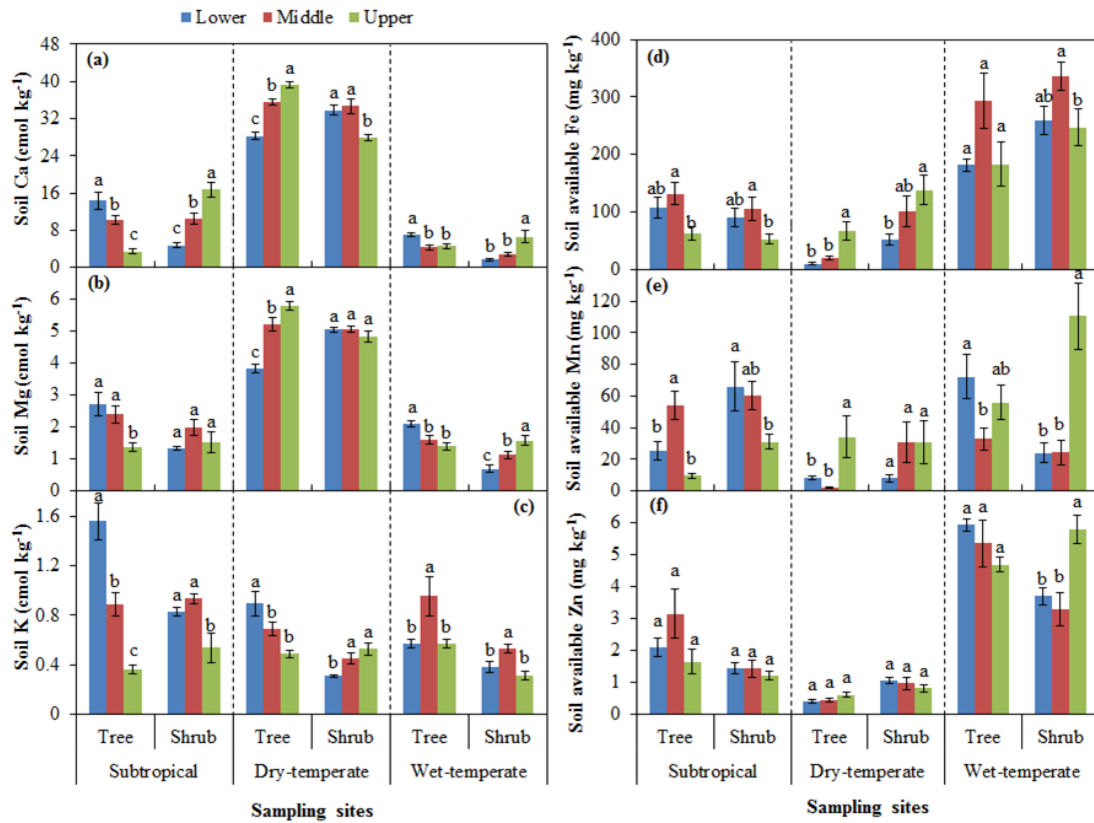



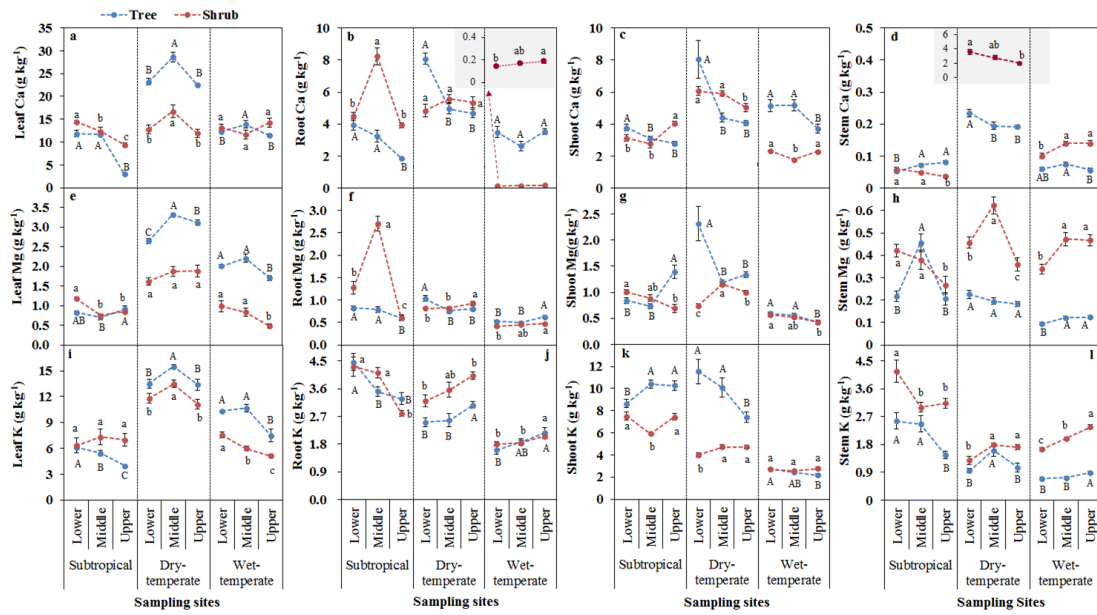
Fig. 2 Concentrations of soil exchangeable base cations of Ca (a), Mg (b) and K (c) and available micronutrients of Fe (d), Mn (e) and Zn (f) at lower and middle elevations as well as at the upper limit of trees or shrubs for each of the three sites. Different letters indicate significant differences among three elevations within treeline or shrubline for each site.

635

Fig. 3 Base cation concentrations of Ca (a, b, c, d), Mg (e, f, g, h) and K (I, j, k, l) in plant tissues of leaf, root, shoot and stem sapwood at lower and middle elevations as well as at the upper limit of trees or shrubs for each of the three sites. Different letters indicate significant differences among three elevations within treeline or shrubline for

640   each site.

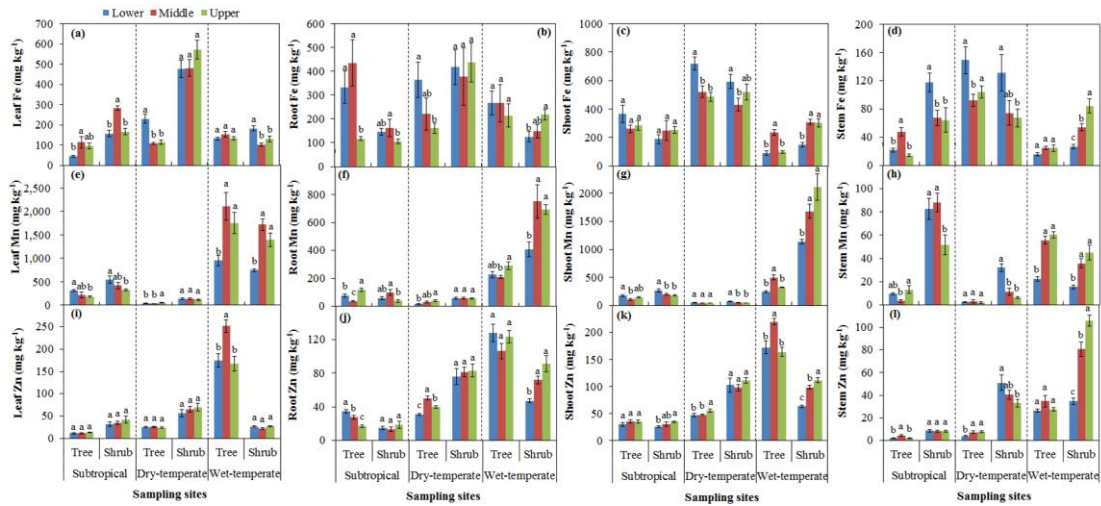



Fig. 4 Micronutrient concentrations of Fe (a, b, c, d), Mn (e, f, g, h) and Zn (I, j, k, l) in plant tissues of leaf, root, shoot and stem sapwood at lower and middle elevations as well as at the upper limit of trees or shrubs for each of the three sites. Different letters indicate significant differences among three elevations within treeline or shrubline for each site.