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 in three climate zones in China. Base cations (Ca, Mg and K) and micronutrients (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). 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 exception 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 in plant tissues.

40   **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 does 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). Nutrient concentrations can

differ among plant tissues as a result of their slow translocation from the roots to the

canopy (van der Heijden et al., 2015), distinct mobility among plant tissues (Warnock,

1970), and occurrence of remobilization during different physiological stages

(Maillard et al., 2015). Quantifying base cation and micronutrient concentrations in

65 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 in soils as well as their variations among plant roots,

leaves, shoots and stem sapwood under changing environmental conditions (Rengel,

70 2007).

The plant distribution and growth along elevational gradients reflect changes in

environmental conditions (Li et al., 2003, 2004, 2006, 2008a,b; Zhu et al., 2012a,b).

Plants growing at high elevation, especially close to their upper limits, are expected to

be highly sensitive to climate change, in particular to global warming (Noble, 1993).

75 Physiological studies of treeline trees have mainly focused on macronutrients such as

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 (Wang et al., 2009).

Two hypotheses have been proposed to explain nutrient accumulation and/or

80 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).

At the alpine treeline, low temperature slows down microbial-mediated litter

85 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). These two

hypotheses are mainly tested on treeline trees, and little attention has been put on

90   other plant life forms, such as shrubline shrubs. Whether trees and shrubs 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

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

systems. Uncertainties still exists whether soil properties 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

100   treeline and shrubline in sub-tropic, dry temperate and wet temperate climate 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. We therefore hypothesized that soil base cation and

micronutrient concentrations increase with increasing elevation because plant uptake

105   decreases more than the supply through weathering and decomposition with elevation.

We also expected that plants of both trees and shrubs 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 plant samples along three

110 elevational gradients from lower elevations up to the alpine treeline or shrubline in

three climate zones in China, and studied the Ca, Mg, K, Fe, Mn, and Zn

concentrations in plant-soil systems.

2 Materials and methods

115 **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,

120 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). Three distinct sites were chosen to find the general

patterns for base cations and micronutrients along elevational gradients across climate

scales rather than to investigate the comparability among study sites. The subtropical

125 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.

130 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 the subtropical Mt., *Picea crassifolia* (elevation range 2540-3250 m) for the dry-temperate Mt., and *Betula ermanii* (elevation range 1700-2030 m) for the wet-temperate Mt.. The shrubs are *Quercus aquifolioides* (elevation range 2840-3590 m) for the subtropical, *Salix gilashanica* (elevation range 3020-3540 m) for the dry-temperate, and *Vaccinium uliginosum* (elevation range 1430-2380 m) for the wet-temperate Mts. The targeted treeline trees and shrubline shrubs are dominant and common species for each study site. The soils from the three sampling sites of subtropical, dry-temperate, and wet-temperate Mts. 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 independent plots (10 m $\times$ 10 m) were selected to serve as 6 replicates on southern slopes with 25 m distance between adjacent plots. Within each plot, 6-10 trees or shrubs of similar height were randomly selected for tissue sampling. Sampling elevations for trees and shrubs were different for each site (Table S1). 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

155   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 subsamples with one subsample being

160   air-dried to constant weight and the other one 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

165   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 of extractable NO_3^--N and NH_4^+-N. Soil Olsen

phosphorus (P) was quantified by colorimetric analysis after extraction with a 0.5 M

170    $NaHCO_3$ 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

suspension. 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

175   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).

180   Plant samples of leaves, roots, shoots and stem sapwood were oven-dried at 60 ℃

for 48 h 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

185   digests were decanted into 50 ml volumetric flasks and the volume was adjusted to 50

ml. The concentrations of Ca, Mg, K, Fe, Mn, and Zn were determined by the AAS

(Shimadzu, Japan).

2.3 Statistical analyses

190   Normality of data was determined using the Kolmogorov-Smirnov test, and

homogeneity of variances using Levene's test. Generalized linear mixed models

(GLMM) 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

195   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. The GLMM

was performed using R version 3.2.3 (http://www.r-project.org).

Within each site, the effect of elevation on measured parameters was determined

200   by multiple comparisons with Duncan's multiple range test for soils and each life

form. Pearson correlation analysis was performed to determine the relationships

between measured parameters using SPSS 16.0 (SPSS, Inc., Chicago, IL, USA). The

soil pH, SOC, TN, ratio of SOC to TN (C:N), NH_4^+ and Olsen P were analyzed as

factors explaining the variability of soil base cation and micronutrient concentrations

205   by structural equation modeling (SEM). Before constructing models, simple linear

regressions between all parameters were examined in order to select factors

explaining the maximum variability. The SEM analyzed the direct, indirect and total

effect of soil parameters on targeted variables. We fitted the models using R statistics

and determined the best-fit models using Akaike information criteria. Statistical

210   significance was accepted at $P < 0.05$ for all the analyses.

3 Results

3.1 Soil pH and SOC

Soil pH was significantly different among elevational positions (Table 1). For both

215   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

220 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

225 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

230 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

235 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

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

240 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..

3.3 Base cations in plants

245   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 with increasing elevation (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 shoots and stem sapwood of shrubs (Fig. 3c,d). For

250   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 shoots of both trees and shrubs at wet-temperate Mt.

255   (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).

260   ### 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 found at the lower elevation in leaves (Fig. 4a), roots (Fig. 4b), shoots (Fig. 4c) and

265   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).

The Mn concentration decreased with increasing elevation in leaves and shoots of

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

275   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 subtropical Mt. (Fig. 4j) and in stem sapwood of shrubs at dry-temperate Mt. (Fig. 4l);

280   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

285   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

290   S2). Negative correlations were found for both Mg and K concentrations between

stems and leaves (both 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 were negatively correlated with soil pH ($p < 0.01$; Table 2, Fig. S2c,d,e),

295   and soil available Fe and Zn were 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). Soil

300   available Fe was negatively correlated with Fe concentrations in shoots and stems

(both $p < 0.01$; Table S3).

According to the SEM analyses, soil pH and TN positively, while C:N negatively

affected exchangeable Ca (Fig. 5a). Exchangeable Mg was positively affected by soil

pH and TN (Fig. 5b). Soil pH, NH_4^+, and Olsen P explained 83% of the variance in

305   exchangeable K (Fig. 5c). Soil available Fe was negatively affected by soil pH and

positively affected by SOC and C:N (Fig. 5d). Soil pH had negative effects on soil

available Fe (Fig. 5d), Mn (Fig. 5e) and Zn (Fig. 5f). A positive effect was detected

for C:N on soil available Fe (Fig. 5d) and for NH_4^+ on both available Mn (Fig. 5e) and

Zn (Fig. 5f). Soil pH indirectly affected exchangeable Ca and Mg as well as available

310   Fe and Mn through changing soil C:N (Fig. 5).

4 Discussion

4.1 Elevational patterns of base cations and available micronutrients in soils and relationships with pH and SOC

315   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

micronutrients indicated that plant uptake of these nutrients did not necessarily

decrease more than nutrient supply at higher elevation due to more open canopies.

320   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. 5 & Fig.

S2). 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

325   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

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

330   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

tree and shrub canopies at dry-temperate Mt. (Table S2). This could indicate species-

and life form-specific effects on soil base cation and micronutrient availabilities.

335   Other soil parameters, such as C:N and extractable NO_3^- and NH_4^+ also

influenced the availability of base cations (Table 2 & Fig. 5). The 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

340   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

345   this study (Table 2). Soil extractable NH_4^+ was also negatively correlated with

exchangeable 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

350   & Fig. 5) 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

355   reason for the positive relationships between SOC and micronutrients (although not always significant, Table 2). While no general patterns were found for distribution of micronutrients under both tree and shrub canopies with elevation, our results suggest that the determinants of soil micronutrient availabilities were predominantly soil pH and SOC concentration, which are reflections of long-term climatic conditions,

360   plant-soil interactions, and biogeochemical processes (Sinsabaugh et al., 2008).

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

365   necessarily contain higher base cation and micronutrient concentrations in their tissues. No general patterns were found for base cations and micronutrients in both trees and shrubs along elevational gradients across the three sites (Fig. 3 & Fig. 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

370   plant tissues (Fig. S3). This suggests that base cation and micronutrient concentrations in plants are influenced by other factors besides elevation-induced changes in 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

375   differences in soil properties (*e.g.*, parent material) among climate zones were larger

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

380   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 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

385   Luo et al. (2015, 2016) were conducted in grassland ecosystems where precipitation

played an essential role in nutrient concentrations in plant-soil systems. 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). Inconsistently elevational patterns in plant

390   nutrient concentrations could also be derived from the fact that individual plant

species reinforced patterns of soil nutrient availabilities in their vicinity causing a

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

395   physiological activities, such as photosynthesis (Welch and Shuman, 1995). Also, it is

an important process in determining plant chemical composition and subsequently

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

400   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

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).

405   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

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

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

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

410   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

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

415   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

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

420   (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

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

425   accumulate more base cations and micronutrients in their tissues to better adapt to

cold environments at higher elevation.

5 Conclusions

We did not find consistent elevational patterns of base cations and micronutrients in

430   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

roles of specific edaphic properties of soil pH, SOC and extractable nitrate and

ammonium in regulating soil base cation and micronutrient availabilities across

climate zones. Soil available base cations and micronutrients were mostly positively

435   correlated with concentrations of base cations and micronutrients in plant tissues,

except for K and Fe. Our results suggest that base cation and micronutrient

concentrations in plants (trees and shrubs) growing at their upper limits are largely

controlled by their availabilities in the soil rather than by plant adaptations to cold

environment at higher elevations.

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Data Availability

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

Author contributions

450 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 Effects (*F* values) 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	19.57**	0.96	4.72*	5.85*	35.76**	3.05	14.73**	20.20**	21.89**	16.48**	0.09	17.14**	42.60**	5.98*	1.42	26.41**	28.90**	2.65	0.10	4.71*	15.84**	2.84	4.44*	5.41*	0.11	2.41	7.45*	18.00**	3.03*	5.10*	2.63	14.45**
E	5.37**	11.96**	0.25	0.61	21.41**	9.10*	6.98**	14.50**	0.00	1.66	0.49	6.82**	3.86*	8.08*	0.67	2.16	3.36*	3.22*	0.15	2.07	0.2	5.08*	3.46	0.66	4.45*	0.91	0.14	1.72	1.47	0.16	1.31	0.40
L×E	15.57**	3.33*	2.40	0.55	8.61**	2.24	8.55**	6.61**	1.11	1.82	7.20*	5.23**	0.37	0.95	1.01	2.20	2.84	0.05	1.69	4.42*	0.36	3.40*	3.72*	0.61	0.15	2.81	1.22	2.37	0.90	1.93	1.34	2.12

650   * 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

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

Table S1 Summary of the main characteristics of three study sites.

Study sites	Position	Soil parent material	Bedrock	MAP (mm)	MAT (°C)	Sampling elevation (m)		Dominant species		Soil type	
						Tree	Shrub	Tree	Shrub		
Subtropical Mt.	102°52′-103°24′E,8.4 30°45′-31°25′N	Moraines sediments	limestone	883 – 1146	2.8 – 5.8	2860, 3290, and 3670	2840, 3160, and 3590	*Abies faxoniana*	*Quercus aquifolioides*	Umbric Cambisols	Cryic
Dry-temperate Mt.	102°58′-103°01′E, 37°14′ -37°20′N	Slope deposit	conglomerate	363 – 550	-4.6 – 1.6	2540, 2870, and 3250	3020, 3250, and 3540	*Picea crassifolia*	*Salix gilashanica*	Calcaric Cambisols	Ustic
Wet-temperate Mt.	126°55′-129°00′E, 41°23′ -42′36″N	Alluvial deposit	trachyte	811 – 1154	-4.8 – 0.3	1700, 1860, and 2030	1430, 2000, and 2380	*Betula ermanii*	*Vaccinium uliginosum*	Andic Cambisols	Gelic

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Table S2 Correlation analyses (*R* values) of soil base cations and available micronutrients with soil physicochemical parameters of treeline or shrubline for each sampling site.

		Treeline						Shrubline					
		Ca	Mg	K	Fe	Mn	Zn	Ca	Mg	K	Fe	Mn	Zn
Subtropical Mt.	pH	**0.47**	0.37	0.54*	0.05	-0.13	-0.35	0.87**	-0.13	-0.56*	-0.60**	**-0.40**	**-0.43**
	SOC	0.31	**0.47**	0.40	0.64**	0.67**	0.78**	**0.43**	0.63**	-0.18	0.15	-0.08	0.36
	TN	0.34	0.49*	0.35	0.57*	0.68**	0.72**	0.55*	0.57*	-0.25	-0.07	-0.19	0.26
	C:N	0.04	0.09	0.27	0.34	0.12	0.27	-0.52*	0.00	0.29	0.72**	0.34	0.23
	NO$_3^-$	0.52*	0.51*	0.63**	0.38	0.11	0.16	0.62**	-0.16	-0.51*	-0.35	-0.17	0.10
	NH$_4^+$	0.08	0.08	-0.21	0.02	**0.43**	0.02	0.00	0.07	0.22	0.13	0.37	0.07
	TIN	0.55*	0.54*	0.51*	0.39	0.33	0.16	0.61**	-0.15	-0.48*	-0.34	-0.14	0.10
	Olsen P	**0.40**	0.67**	0.54*	0.29	0.31	0.49*	-0.21	-0.08	0.37	0.24	-0.09	-0.28
Dry-temperate Mt.	pH	-0.85**	-0.79**	0.63**	**-0.43**	-0.12	-0.40	-0.07	0.07	0.38	0.35	0.49*	-0.13
	SOC	0.59*	0.60**	-0.36	0.08	-0.09	0.22	0.64**	**0.45**	-0.37	-0.20	-0.25	0.10
	TN	0.95**	0.94**	-0.72**	0.63**	0.38	**0.46**	0.01	0.09	0.22	-0.03	-0.35	0.01
	C:N	-0.52*	-0.49*	0.53*	-0.70**	-0.57*	-0.32	0.79**	0.48*	-0.63**	-0.21	-0.04	0.07
	NO$_3^-$	-0.48*	-0.38	0.32	-0.18	-0.12	-0.39	-0.51*	-0.01	0.32	0.26	0.28	-0.05
	NH$_4^+$	0.19	0.30	-0.19	0.24	0.36	0.32	**0.46**	0.13	0.03	-0.33	-0.30	-0.09
	TIN	**-0.43**	-0.31	0.27	-0.12	-0.04	-0.30	-0.26	0.10	**0.44**	0.04	0.10	-0.14
	Olsen P	0.48*	0.53*	-0.28	0.20	0.12	0.47*	-0.33	-0.25	0.67**	**0.46**	0.39	-0.14
Wet-temperate Mt.	pH	0.03	-0.22	0.13	-0.57*	**0.41**	0.15	0.66*	0.49*	**-0.46**	-0.53*	0.65**	**0.41**
	SOC	-0.09	0.15	0.56*	0.91**	-0.57*	0.07	0.67**	0.83**	0.36	0.61**	**0.41**	0.54*
	TN	**0.43**	0.65**	**0.44**	0.71**	-0.32	**0.43**	0.78**	0.88**	0.22	0.47*	0.53*	0.55*
	C:N	-0.70**	-0.58*	**0.42**	0.66**	-0.61**	**-0.42**	-0.48*	-0.50*	0.09	0.06	**-0.40**	-0.24
	NO$_3^-$	-0.07	-0.20	-0.32	-0.21	0.18	-0.12	-0.25	-0.32	0.02	-0.07	-0.30	-0.16
	NH$_4^+$	0.09	0.19	0.61**	-0.07	-0.29	0.49*	-0.29	**-0.43**	-0.09	-0.32	-0.22	-0.20
	TIN	-0.03	-0.11	-0.08	-0.23	0.06	0.07	-0.28	-0.37	-0.02	-0.15	-0.29	-0.18
	Olsen P	0.01	0.12	0.49*	0.18	0.04	**0.44**	0.27	0.48*	0.49*	0.17	0.20	0.34

* and ** indicate significant level at $P < 0.05$ and 0.01, respectively. Bold type represents $P < 0.1$.

Table S3 Correlation analyses for base cations and available micronutrients in soil
and plant tissues of leaf, root, shoot and stem sapwood across sampling sites, plant life
form and elevation position.

Base cations and micronutrients	Correlation analyses				
Ca		Soil Ca	Leaf Ca	Root Ca	Shoot Ca
	Leaf Ca	0.60**			
	Root Ca	0.56**	0.37**		
	Shoot Ca	0.56**	0.37**	0.54**	
	Stem Ca	0.56**	-0.01	0.24*	0.43**
Mg		Soil Mg	Leaf Mg	Root Mg	Shoot Mg
	Leaf Mg	0.66**			
	Root Mg	0.10	-0.09		
	Shoot Mg	0.44**	0.44**	0.21*	
	Stem Mg	0.10	-0.41**	0.17	-0.09
K		Soil K	Leaf K	Root K	Shoot K
	Leaf K	-0.06			
	Root K	0.34**	-0.08		
	Shoot K	0.32**	0.07	0.44**	
	Stem K	0.21*	-0.46**	0.55**	0.20*
Fe		Soil Fe	Leaf Fe	Root Fe	Shoot Fe
	Leaf Fe	-0.17			
	Root Fe	-0.07	0.37**		
	Shoot Fe	-0.43**	0.38**	0.27**	
	Stem Fe	-0.39**	0.35**	0.13	0.53**
Mn		Soil Mn	Leaf Mn	Root Mn	Shoot Mn
	Leaf Mn	0.30**			
	Root Mn	0.28**	0.67**		
	Shoot Mn	0.35**	0.61**	0.89**	
	Stem Mn	0.32**	0.46**	0.19*	0.22*
Zn		Soil Zn	Leaf Zn	Root Zn	Shoot Zn
	Leaf Zn	0.52**			
	Root Zn	0.57**	0.69**		
	Shoot Zn	0.62**	0.86**	0.88**	
	Stem Zn	0.44**	0.11	0.53**	0.48**

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

Figures

670

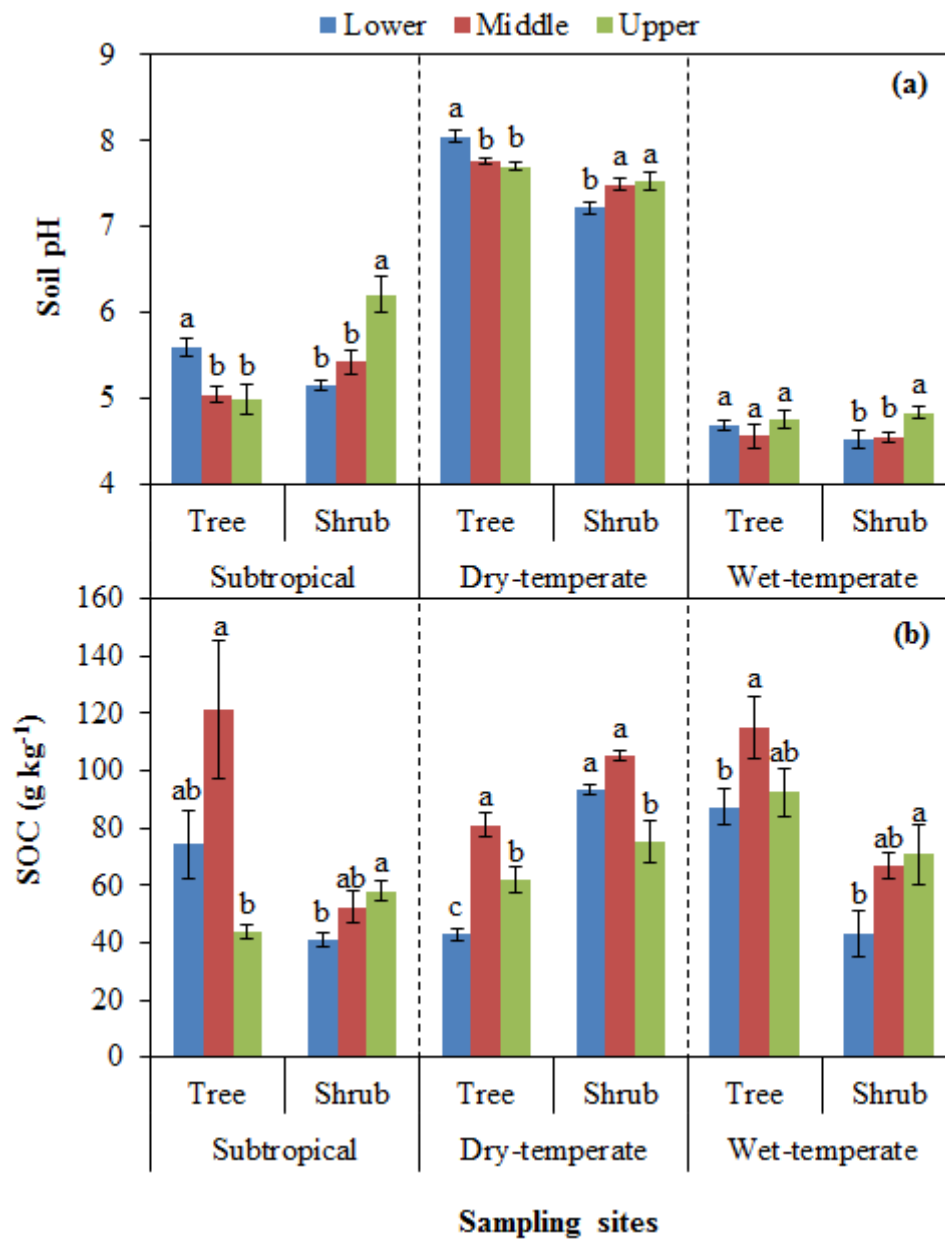



Fig. 1 Soil pH values (a) and soil organic carbon (SOC) concentration (b) at lower and middle elevations as well as at the upper limit of treelines or shrublines for each of the three sites. Different letters indicate significant differences among three

675 elevations within treeline or shrubline for each site.

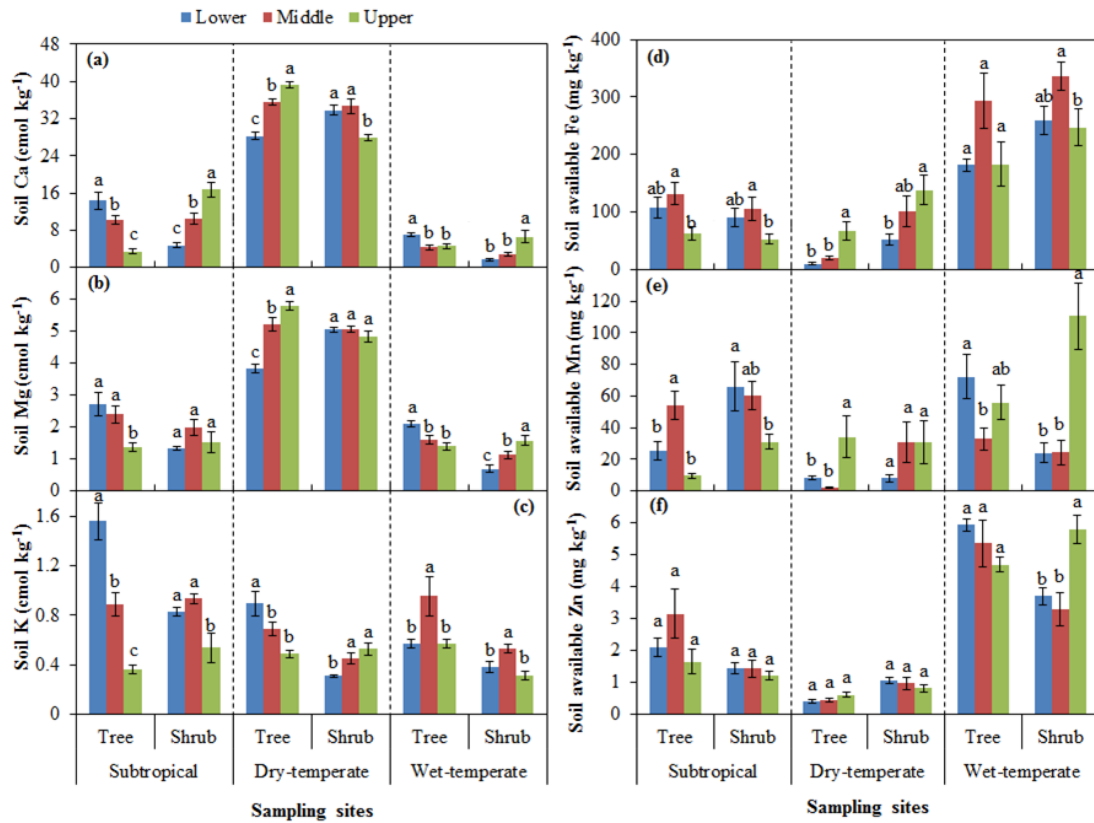



Fig. 2 Concentrations of soil exchangeable base cations of Ca (a), Mg (b) and K (c) and available micronutrients of Fe (d), Mn (e) and Zn (f) at lower and middle elevations as well as at the upper limit of trees or shrubs for each of the three sites. Different letters indicate significant differences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

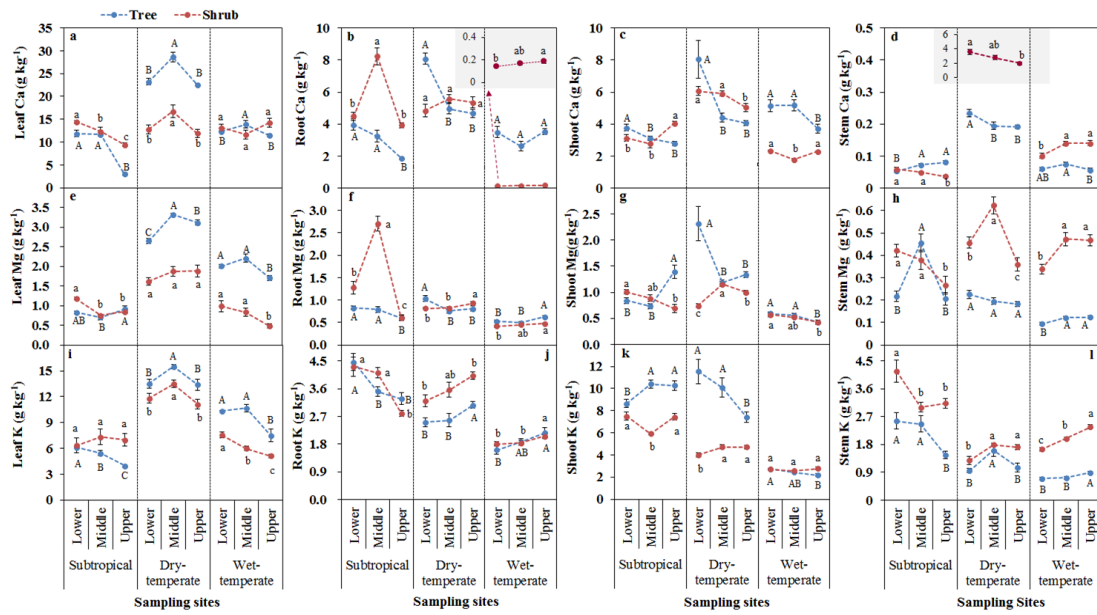



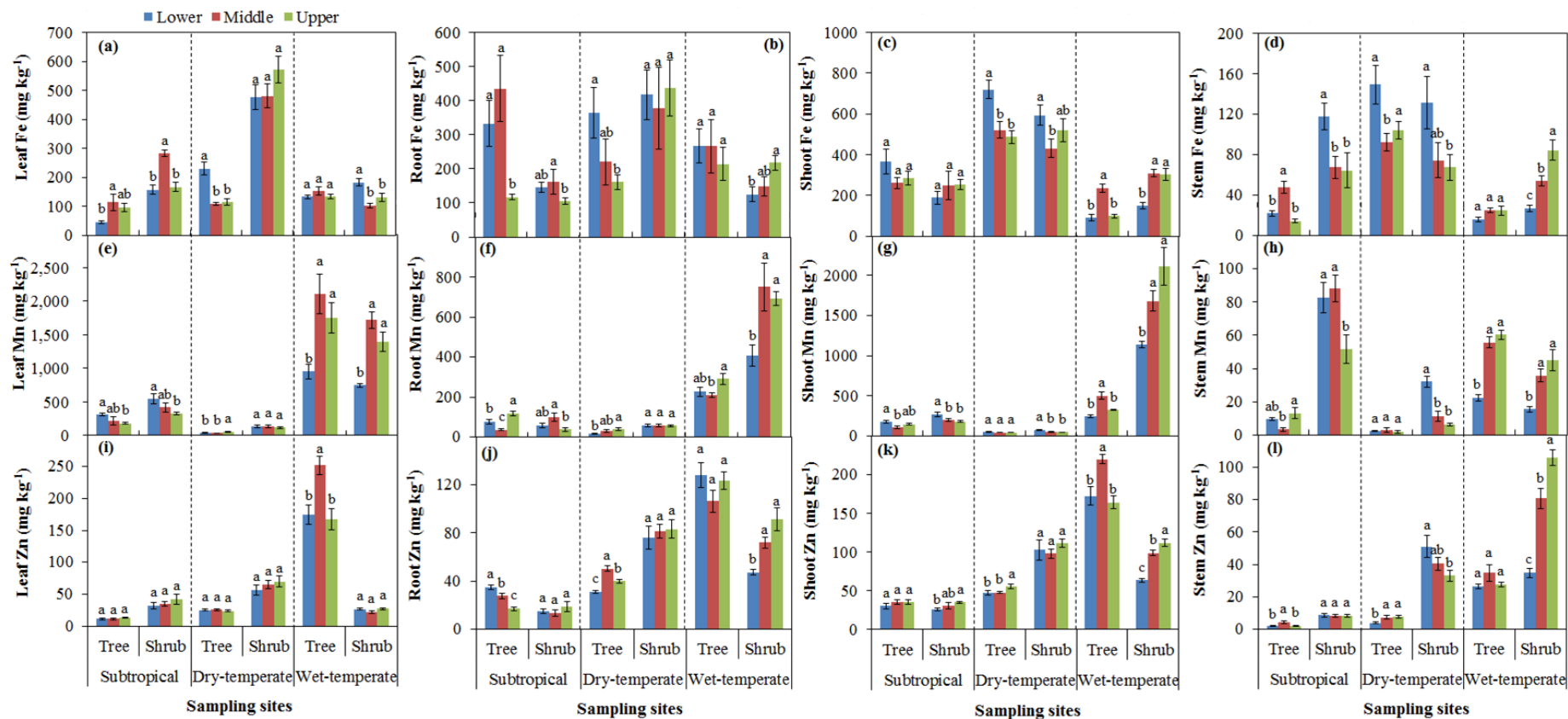
Fig. 3 Base cation concentrations of Ca (a, b, c, d), Mg (e, f, g, h) and K (I, j, k, l) in plant tissues of leaf, root, shoot and stem sapwood at lower and middle elevations as well as at the upper limit of trees or shrubs for each of the three sites. Different letters indicate significant differences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

690

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.

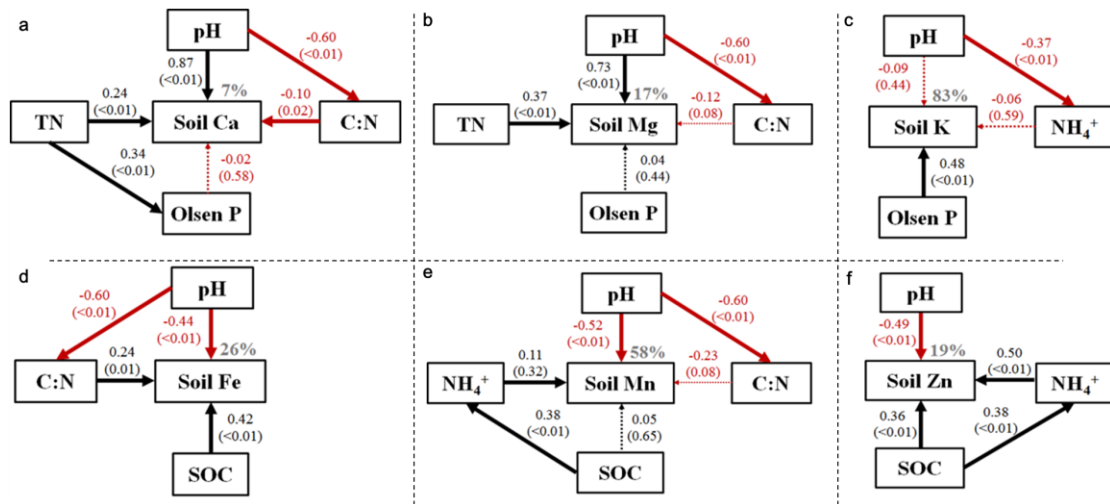



Fig. 5 The results of structural equation modelling of the effect of soil parameters on soil exchangeable base cations of Ca, Mg and K, and available micronutrients of Fe, Mn and Cu. Arrows indicate positive (black) and negative (red) effects. Solid and dotted lines represent significant and non-significant relationships, respectively. The number adjacent to each arrow is the standardized path coefficient with corresponding *p* value between brackets.

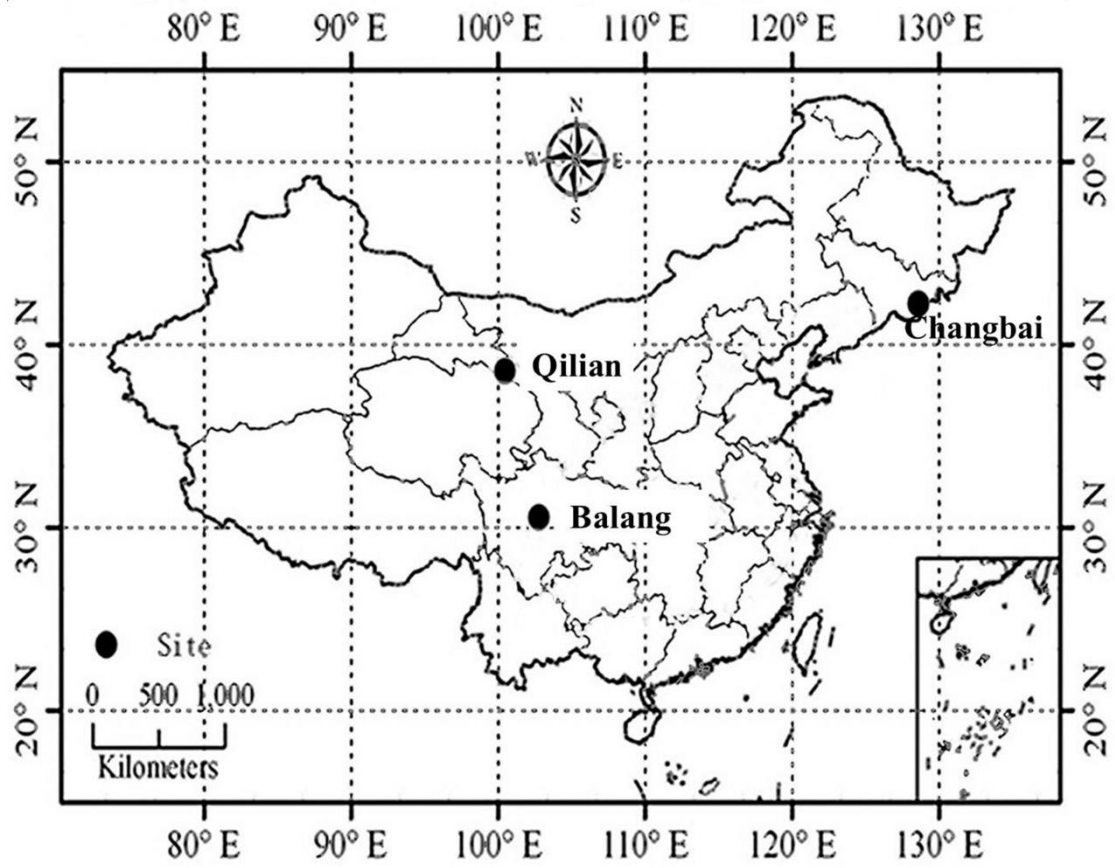



Fig. S1 Location of three sampling sites of Balang (subtropical Mt.), Qilian

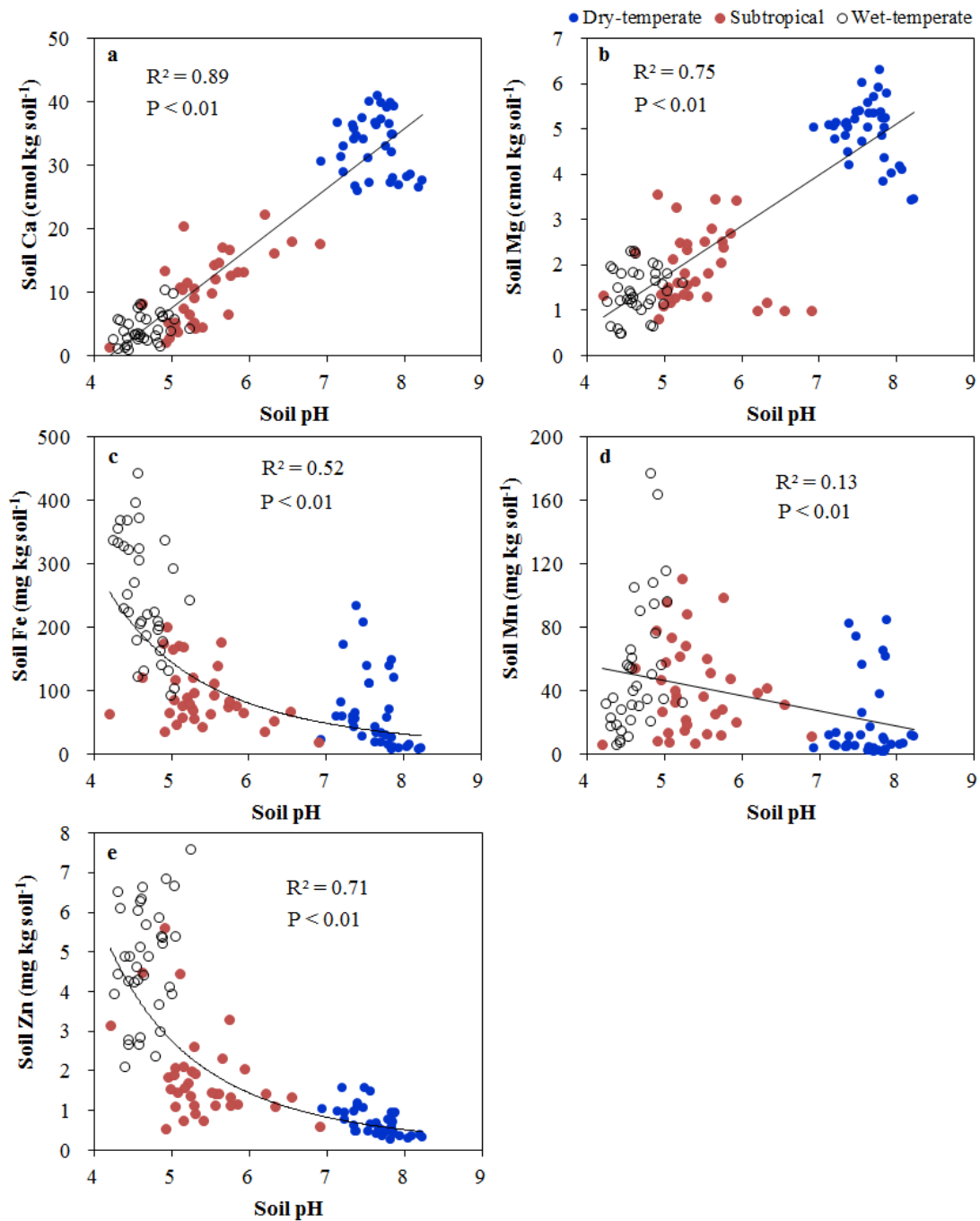
(dry-temperate Mt.) and Changbai (wet-temperate Mt.).

705

Fig. S2 Correlation analyses between soil pH and soil exchangeable base cations of

Ca (a) and Mg (b) and available micronutrients of Fe (c), Mn (d) and Zn (e) under

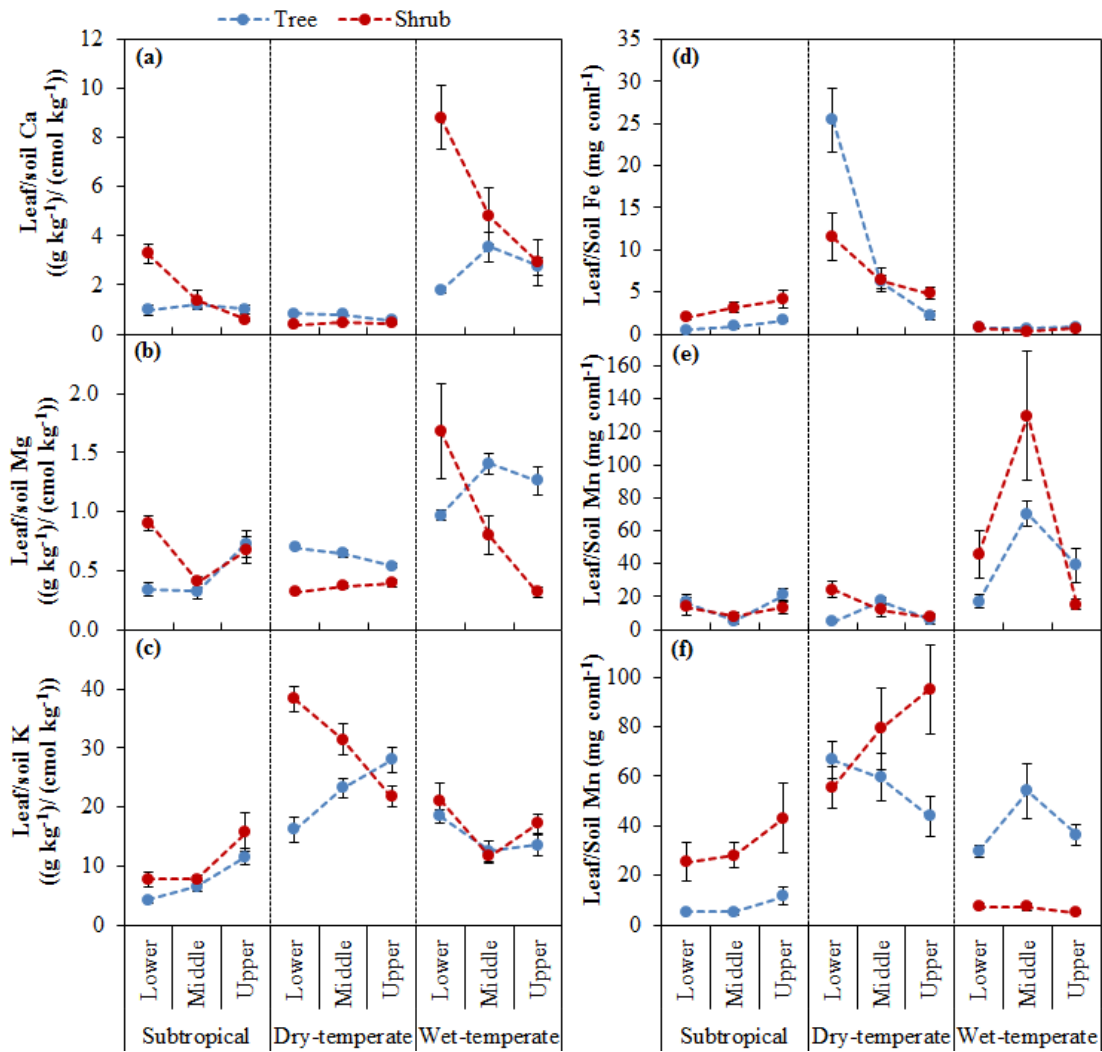
trees and shrubs across three sampling sites.

710

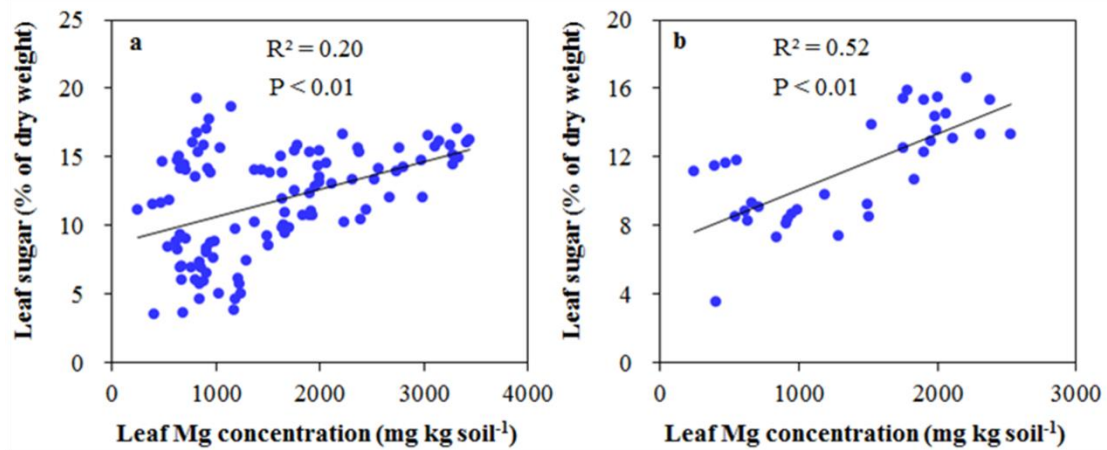
Fig. S3 The ratio of nutrient concentrations of Ca (a), Mg (b), K (c), Fe (d), Mn (e) and Zn (f) in plant leaves to their availabilities in soils at lower and middle elevations as well as at the upper limit of trees or shrubs for each of the three sites.

715

Fig. S4 Correlation analyses between Mg concentration and soluble sugar contents across three sampling sites (a) and at wet-temperate Mt. (b).