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REGULAR ARTICLE

Evidence of elemental homeostasis in fine root and leaf tissues of saplings across a fertility gradient in tropical montane forest in Hainan, China

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Abstract
Aims For plants, elemental nutrients are important belowground resources that sustain growth and survival. To understand how tropical plant nutrient status responds to environmental variation, we asked whether concentrations of nutrients in root and leaf tissues track gradients in soil nutrient concentrations and if tissue nutrient concentrations respond independently or in concert to soil nutrient concentrations.
Methods We measured soil nutrient concentrations of rhizosphere soil and root and leaf tissue elemental concentrations of saplings from 14 Angiosperm families in montane tropical forest of Jianfengling, China. Using mixed-effects models, we modeled the nutrient concentration of plant tissues as a function of soil resources.
Results Of fourteen elements measured, seven —nitrogen, boron, phosphorus, potassium, manganese, copper and zinc— increased in concentrations in root and leaf tissues with higher soil nutrient availability; two decreased —aluminum and carbon; three were invariant —magnesium, sulfur, and calcium; and two —sodium and iron— showed contrasting patterns between leaves and roots. Eight elements necessary to leaf physiological function, but also used in root functioning —nitrogen, boron, magnesium phosphorus, sulfur, potassium, calcium, manganese— were more concentrated in leaves than roots. Additionally, most elements showed tradeoffs in concentrations between roots and leaves. Plant lineage (i.e. family) explained very little of the variation about this overall trend.
Conclusions Overall, increases in tissue nutrient concentrations with soil fertility were subtle if present at all. Thus, we conclude that tissue nutrients of juvenile tropical trees have a high degree of elemental homeostasis with local-scale soil nutrient content in Jianfengling.

Keywords Plant nutrient analysis · Leaf chemistry · Root chemistry · Stoichiometry · Tropical forest · Jianfengling · Responsible Editor: Philip John White

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Introduction

The study of the mineral nutrition of plants has long been a hallmark of the agricultural sciences (Aulie 1974; Chapin 1980; Marschner 2012); however, understanding how wild plants use and store nutrients is becoming increasingly important as humans continue to alter

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biogeochemical cycles (Cleland and Harpole 2010; Elser et al. 2007; Hobbie 2015). Plant growth and proper cellular functioning are directly dependent on nutrient uptake from the soil, and nutrient deficiencies can lead to poor plant health and reduced performance (Aerts and Chapin 1999; Chapin 1980; Marschner 2012), which can affect ecosystem processes. Plants respond to changes in soil nutrient fertility, and they regulate their response based upon the flow of energy, water, and nutrients within the plant (Pons et al. 1998). Therefore, from an ecological perspective, quantifying the nutrient status of plant organs can inform about whole plant nutrition and physiology (Chapin 1980; Kramer and Kozlowski 1979; Pons et al. 1998).

Organismal stoichiometry, or the study of the elemental composition of organisms (Elser et al. 2000a, 2000b), examines how the chemical make-up of organisms reflects the functioning of their biological parts within the environment. Stoichiometric theory contends that organisms generally have fixed nutritional requirements that result in a consistent stoichiometry (i.e., elemental composition). Although the stoichiometry of plant tissues is more flexible and tends to reflect their abiotic environment to a greater degree than in animals (Elser et al. 2000b), some stoichiometric balance between the elemental concentration and composition in plant tissues and their growth environment is expected, in order to maintain optimal physiological functioning; this balance is termed elemental homeostasis (Sterner and Elser 2009; Sterner and Elser 2002), and the degree to which it operates in wild plants, and varies with environment or plant lineage, is not entirely known. As such, elemental homeostasis remains the testable null hypothesis for applied stoichiometric studies (Sterner and Elser 2009, Fig. 1).

Fourteen to twenty elements are essential for plant growth (depending on the species of plant and definition used for essentiality) (Table 1). From a plant nutrition perspective, these elements are best understood when divided into four groups based on chemical behavior and function (Mengel and Kirkby 2001; Marschner 2012). Group 1 elements (*carbon*, hydrogen, oxygen, *nitrogen*, and *sulfur*)[1] are taken up as ions from the soil solution or as gases from the atmosphere. They are the principal constituent elements of organic matter assimilated via oxidation-reduction reactions in plant cells. Group 2 elements (*phosphorus*, *boron*, and silicon)[1] are obtained as inorganic anions or acids from the soil solution and are found in similar forms within plant cells (e.g., phosphate). Group 3 elements (*potassium, sodium, calcium, magnesium, manganese.* and chlorine)[1] are taken up from the soil in ionic form and occur in cells as cationic compounds or chelates. Lastly, group 4 elements (*iron, copper, zinc,* and *molybdenum*) are obtained as ions or chelates from the soil solution and are used in the cell as ions that engage in various cellular functions including electron transport. The purpose of nutrients in groups 3 and 4 depends on their oxidation state to create osmotic potentials and ionic gradients, influencing structural changes to enzymes, and mediating cellular redox reactions (Marschner 2012).

Nitrogen (N) is arguably the most studied element in terms of plant function and growth (Enríquez et al.

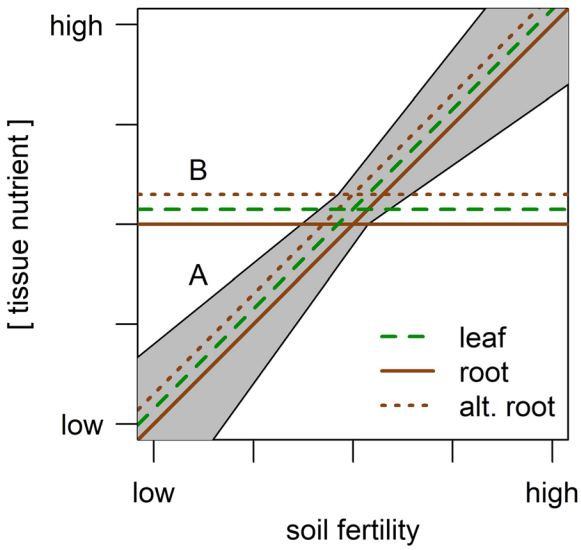

Fig. 1 Competing hypotheses regarding relationships of tissue nutrient concentrations (y-axis) to soil fertility (x-axis). Note that x and y-axes should be logarithmic when analyzing actual data (Sterner and Elser 2002). Hypothesis A: tissue nutrient concentrations track soil fertility in a linear fashion; for example, leaf and root tissue N concentration increases in direct proportion to soil available N. This is the null hypothesis of ecological stoichiometric theory (i.e., the "you are what you eat" hypothesis) (Sterner and Elser 2002), where plant tissue chemistry changes in lockstep with its chemical availability in the soil resource. Hypothesis B: plants maintain homeostasis in tissue nutrient concentrations, independently of resource availability in the soil (i.e., no change in tissue nutrient concentration). Within that hypothetical framework, root tissue nutrient concentrations may either be less than (solid brown line) or greater than (dotted brown line) the tissue nutrient concentrations of leaves (dashed green line). The gray shaded areas show that the slope of the relationship in hypothesis A may vary (i.e., relationships of leaf and root tissue nutrient concentrations may create intersecting lines)

[1] Italicized elements are the ones measured in this study.

Table 1 Elemental information, known functions within the plant, and hypothesized relationships with gradients of soil fertility for 15 essential plant nutrients, which were analyzed in root and leaf tissue of juvenile trees from Jianfengling, Hainan Island, China

Element	carbon (C)	nitrogen (N)	boron (B)	sodium (Na)	magnesium (Mg)	aluminum (Al)	phosphorus (P)	sulfur (S)
Atomic Number	6	7	5	11	12	13	15	16
Usual Oxidation State(s)	–4, –3, –2, –1, +1, +2, +3, +4	–3, +3, +5	3	1	2	3	–3, +3, +5	–2, +2, +4, +6
Isotopes	^{12}C, ^{13}C	^{14}N, ^{15}N	^{10}B, ^{11}B	^{23}Na	^{24}Mg, ^{25}Mg, ^{26}Mg	^{27}Al	^{31}P	^{32}S, ^{33}S, ^{34}S, ^{36}S
Common environmental forms	carbonates	nitrates, nitrites, ammonium	borax, boric acid, kernite, ulexite, colemanites, borates	sodium chloride, sodium carbonate, sodium borate, sodium nitrate, sodium sulfate	magnesium sulfate,	aluminum hydroxide, aluminum oxide, aluminum silicates	phosphates, organophosphates, phospho mono- and di-esters.	silfides, sufates.
Associated physiological function in leaf tissue	Carbohydrates, simple sugars, lignin, and hemicellulose compounds for plant structure (e.g., cell walls), herbivory defense.	maximum photosynthetic rate, maximum rate of carboxylation, RuBP content, Rubisco content.	Helps build sugars following photosynthesis, interacts with K to regulate stomatal guard cell function, involved with nitrogen for metabolism and protein formation.	Enhances nitrate assimilation, regulation of leaf area and stomatal density, related to leaf chlorophyll content.	Photosynthetic rates, chloroplast & photosystem function, leaf enzyme regulation, leaf longevity.	Limited functionality, but at high concentration, Al reduces chlorophyll function and photosynthetic electron flow, membrane and protein functioning	Constrains A_{max}-leaf N relationships, acts as the principal element in ATP, involved in enzyme reactions, used in healthy ribosome function and DNA and RNA transcription/ translation.	Involved in cysteine synthesis in the chloroplast, associated with bundle sheath cell function in C4 plants.
Associated physiological function in root tissue	Translocation to other plants, mycorrhizae, root nodules, root cell membrane health.	Nitrogen uptake by roots, root protein and enzyme content.	Adventitious rooting (potentially counteracting the adverse effect of phenolics), and root development.	Enhances nitrate uptake by roots and nitrate assimilation in leaves. Interacts with K in root ATPase regulation.	Important for cell division and root growth interacts with other nutrients to regulate nutrient transport across membranes, associated with root apoplast function, involved in phosphatase	Interacts primarily with the root cap, at high concentration can reduce root elongation, can aid in reducing the toxicity of other metals (Fe, Mn), can reduce fungal parasitism of roots.	Involved in regulation of root architecture P deficiency leads to root elongation and branching (i.e., increasing acquisitiveness), associated with mycorrhizal colonization of roots and	Inorganic sulfate uptake in roots is associated with cotransport of proteins (ATPase activity).

Table 1 (continued)

Element	carbon (C)	nitrogen (N)	boron (B)	sodium (Na)	magnesium (Mg)	aluminum (Al)	phosphorus (P)	sulfur (S)
					production (e.g., alpha phosphatase).		regulation of rhizosphere microbiome, root enzyme activity (especially phosphatase).	
Associated whole-plant physiological function	Growth and tissue production, resistance to drought.	building block of amino acids and (soluble) proteins, a major component of DNA.	Unclear, but potentially: sugar transport, cell division, cell wall construction, lignification, carbohydrate, RNA and phenol metabolism, membrane function, and respiration.	Growth stimulation (depending on species sensitivity); can cause physiological stress at high concentrations, phloem mobility, cell expansion, plant water balance, proper tonoplast and vacuole function.	Starch synthesis, and maintenance of starch sinks, important for the aggregation of ribosomal subunits and protein synthesis .	Toxic at high concentrations. In Al toxicity, Al interferes with the uptake and transport of essential cations (Cu, Zn, Ca, Mg, Mn, K, P, and Fe), reduces photosynthesis, respiration, and protein synthesis.	Cellular health (phospholipid bilayer), seed production (phosphorylated alcohol), nucleic acids, enzymatic activity, regulation of plant metabolism, cytoplasmic function, involved in root-shoot ratio regulation, hormonal signaling.	Amino acid function (specifically, cysteine and methionine), protein cofactor function and protein synthesis, cystol function, photosynthesis, respiration.
Relationship to soil fertility or pH and explanation	↓/↔ Plant tissue carbon is thought to be largely invariant to soil fertility but may decrease in less fertile soils if allocation to roots is increased, or more liable carbon is being exchanged to the rhizosphere for nutrients.	↑ Whole plant and leaf nitrogen increase with soil nitrogen. Root nitrogen concentrations should reflect soil availability of ingoranic forms, and root enzyme production and nitrogen cycling rates.	↔ Boron deficiency may result when concentrations are low in the soil (i.e., <20 ppm). Boron weakly adsorbs to the soil, but can undergo passive uptake, once in soluble form. It likely never limits plant growth alone. Tissue	↑ In the absence of salt stress, sodium concentrations in plant tissues should be low in low fertility soils, but may increase as soil fertility increases given sodium's ability to enhance nutrient uptake.	↑ Magnesium deficiency in tissues may increase with decreasing soil fertility and increasing soil acidity, due to the presence of competing divalent cations in acidic, nutrient-depleted soils (aluminum, calcium, sodium).	↓ Concentrations of aluminum in plant tissues should be low but have the potential to increase with decreasing soil fertility and increasing soil acidity.	↑ Soil phosphorus often limits plant growth, so it should increase in plant tissues with availability in the soil. However, species differ drastically in their affinities for phosphorus, seeking to maintain inorganic phosphorus	↔ Globally, sulfur deficiency in soils is widespread. Plants can only utilize inorganic sulfur from the soil, which is always in low supply. Therefore, no relationships between tissue concentrations and soil

Table 1 (continued)

Element	carbon (C)	nitrogen (N)	boron (B)	sodium (Na)	magnesium (Mg)	aluminum (Al)	phosphorus (P)	sulfur (S)
			concentrations should be consistent across soil gradients.				homeostasis in the cytoplasm. Mechanisms exist to increase or regulate phosphorus uptake from the soil, or store phosphorus in plant tissue.	gradients are known.
Sources	(Dietze et al. 2014; Kozlowski 1992; Ma et al. 2018; Silver and Miya 2001)	(Evans 1989; Field and Mooney 1986; Fitter and Hay 2012; Jackson et al. 1997; Lambers and Poorter 1992; Silver and Miya 2001; Valverde--Barrantes et al. 2007; Wright et al. 2004)	(see table 2 in Hull 2002; Kutschera and Niklas 2017; Lewis 2019; Middleton et al. 1978)	(Garg et al. 1993; Hampe and Marschner 1982; Subbarao et al. 2003)	(Wilkinson et al. 1990; Shaul 2002)	(Delhaize and Ryan 1995; Kahle 1993; Roy et al. 1988)	(Lambers et al. 2006; Reich et al.2009a; Schachtman et al. 1998; Shen et al. 2011; Smith 2001; Turner 2008; Turner et al. 2018; White and Hammond 2008)	(Anderson 1990; Balk and Pilon 2011; Lewandowska and Sirko 2008)

Element	potassium (K)	calcium (Ca)	manganese (Mn)	iron (Fe)	copper (Cu)	zinc (Zn)	molybdenum (Mo)
Atomic Number	19	20	25	26	29	30	42
Usual Oxidation State(s)	1	2	+2, +4, +7	+2, +3, +6	2	2	+4, +6
Isotopes	^{39}K, ^{41}K	^{40}Ca, ^{42}Ca, ^{43}Ca, ^{44}Ca, ^{46}Ca	^{55}Mn	^{54}Fe, ^{56}Fe, ^{57}Fe, ^{58}Fe	^{63}Cu, ^{65}Cu	^{64}Zn, ^{66}Zn, ^{68}Zn, ^{68}Zn, ^{70}Zn	^{92}Mo, ^{94}Mo, ^{95}Mo, ^{96}Mo, ^{97}Mo, ^{98}Mo
Common environmental forms	potassium chloride,	calcium oxides, calcium silicates, calcium carbonate, calcium chloride, calcium phosphate	pyrolusite, rhodochrosite	iron oxides, ferrosic hydroxide, siderite, iron chelates	ionic forms (CU^{2+}), cupric oxide, cuprous oxide, copper sulfate,	zinc oxide, zinc chloride, zinc phosphate, zinc sulfide.	molybdenite, molybdenum oxide, wulfenite ($PbMoO_4$), powellite ($Ca(MO,W)O_4$)

Table 1 (continued)

Element	potassium (K)	calcium (Ca)	manganese (Mn)	iron (Fe)	copper (Cu)	zinc (Zn)	molybdenum (Mo)
Associated physiological function in leaf tissue	Ion transport in chloroplasts and mitochondria, Glucose synthesis, ATP synthesis, photosynthesis, stomatal behavior via regulation of turgor pressure in guard cells.	Stabilizes cell membranes through interactions with phospholipid head, cell wall formation, cystol signaling, enzymatic activation.	Involved in photosynthesis – the manganoprotien of photosystem II is involved in the water-splitting process of photosynthesis, involved in cell wall function and metal transport across cell membranes.	Key to the energy transduction pathway in photosynthesis, a principle reducing agent in mitochondrial and chloroplast activities.	Chloroplast function (light reactions), involved in leaf photorespiration (via polyphenol oxidase), enzyme activation (cofactor behavior, interchangeable with Zn).	Chloroplast and mitochondrial function, involved in thylakoid lumen and chloroplast stroma activity, associated with increased mesophyll conductance in woody plants.	Associated with peroxisome function, mitochondrial function, leaf enzyme activity (e.g., aldehyde and xanthine oxidases).
Associated physiological function in root tissue	Key to establishing root membrane potentials for active ion transport into the tonoplast, cation-anion balance, response to physiological stress (e.g., drought, severe nutrient limitation).	Root signaling in ionic uptake processes, regulation of root ion carries and channels, root elongation.	Linked to other divalent cation (Ca, Mg, Fe, Cu, Zn) uptake in roots (via transport proteins).	Essential to root phosphatase activity (e.g., purple acid phosphatases), interacts with ion transport of other divalent metals through ion channels.	Associated with nitrogen fixation (i.e., *Rhizobium* root nodule).	Essential to phosphatase activity and other root enzymatic activity.	Associated with nitrogen fixation in root nodules (i.e., *Rhizobium* enzyme function – nitrogenase), interacts with iron and sulfur uptake in roots.
Associated whole-plant physiological function	Xylem function, enzyme activation, protein synthesis (ribosomal function), osmotic function (cystol / vacuole stoichiometry), a counter ion for nitrate transport in xylem, key to nucleic acid construction.	Regulation of intercellular water flow, transpiration, endoplasmic reticulum function.	Functionally similar to magnesium, involved in enzyme synthesis and function, involved in most enzyme-activated cellular reactions, such as phosphorylation, decarboxylation, reduction and hydrolysis reactions.	Toxic at high concentrations, involved in nitrate reductase enzymatic activity, involved in increased tolerance of metals.	Xylem function, associated with proper enzyme function (e.g., ascorbic acid), mitochondrial respiration.	Xylem function, cytoplasm function, crucial to the active center of carbonic anhydrase activity, which interconverts CO_2 and carbonate, regulates gene expression (e.g., Zinc finger proteins).	Involved with the reduction of nitrate through processes that provide activation energy, associated with the function of the azotase enzyme.
Relationship to soil fertility or pH and explanation	↑ Potassium uptake in roots in biphasic, meaning both passive and active uptake are possible	↑ Calcium uptake and concentration in plant tissues is highly dependent on soil pH and should	↔ Due to its exchangeability for other soil cations, relationships of manganese are	↔ In tropical forest soils, iron is non-limiting; however, most of the iron in tropical	↔ Tissue copper concentrations are suspected to be far higher than needed for	↓ Zinc accumulation typically occurs to a higher degree in nutrient-poor soils, accumulation is	↔ Alkaline conditions favor molybdenum liberation from soils, but plant

Table 1 (continued)

Element	potassium (K)	calcium (Ca)	manganese (Mn)	iron (Fe)	copper (Cu)	zinc (Zn)	molybdenum (Mo)
	and depend on the difference in concentration of potassium in the rhizosphere and root tissue. Tissue concentrations should, therefore, reflect soil availability, increasing with soil fertility.	decrease with decreasing soil pH and fertility. Calcium concentrations in the soil affect Na and K uptake.	likely invariant to soil fertility. Manganese toxicity may develop with decreasing soil fertility and increasing soil acidity.	soils (Ferric ionic forms, iron oxides. etc.) is inaccessible to plants and must undergo reduction (to Ferrous ion) at the root surface, depending on soil pH. Along soil gradients of the same soil type, tissue concentrations of iron should be consistent.	physiological functioning; therefore, no relationship with soil fertility is expected.	typically higher in roots than in leaves and should be decreased with soil fertility.	uptake increase with soil acidity. Molybdenum accumulation is increased by phosphate and sulfate addition. Therefore, association with soil fertility unclear.
Sources	(Cakmak 2005; Maathuis and Sanders 1996; Mengel 2016)	(Epstein 1961; Gilliham et al. 2011; Kahle 1993; Marschner 1991; Marschner 2012; White and Broadley 2003)	(Andresen et al. 2018; Burnell 1988; Edwards and Walker 1983; Marschner 1991; Mukhopadhyay and Sharma 1991)	(Andresen et al. 2018; Brown 1978; Jeong and Guerinot 2009; Kahle 1993; Vose 1982)	(Andresen et al. 2018; Kahle 1993; Marston 1952; Yruela 2005)	(Andresen et al. 2018; Broadley et al. 2007; Kahle 1993; Longnecker and Robson 1993)	(Andresen et al. 2018; Bittner 2014; Kahle 1993; Marston 1952; Zimmer and Mendel 1999)

For typical concentrations of elements in plant tissues see Table 3.1 in Fitter and Hay (2012). Much of the general information in this table was drawn from (Kramer and Kozlowski 1979; Marschner 2012; Mengel and Kirkby 2001). Literature-based hypothetical relationships for plant tissue concentrations of each element with an increase in soil fertility are represented with arrows to denote increasing tissue concentrations (↑), decreasing tissue concentrations (↓), or no change (↔). Note that in this table we include Molybdenum, however concentrations were undetectable for the majority of tissue samples that we measured, so it was excluded from our analyses

1993, Güsewell 2004, Elser et al. 2000b, see references in Table 1). Leaf tissues are richer in nitrogen than stems and roots (Pregitzer et al. 1997) because of the many specialized proteins and enzymes required for photosynthesis (Pons et al. 1998; Reich and Oleksyn 2004). Moreover, leaf nitrogen generally is higher in species with fast life-history strategies and high rates of photosynthesis and carboxylation, because they have more proteins (e.g., thylakoid proteins) and enzymes (e.g., Rubisco) that are nitrogen-rich than species with slower life-history strategies (Field and Mooney 1986; Lambers and Poorter 1992; Reich et al. 1992; Wright et al. 2004).

Variation in root nitrogen concentration appears to be more related to construction costs in roots than acquisition performance (Maire et al. 2009; McCormack and Iversen 2019; Bergmann et al. 2020); nevertheless, concentrations tend to reflect inorganic N-availability in the soil and variation in life history and root functional strategies. For example, root nitrogen concentrations are related to rates of root respiration (Makita et al. 2009; Paradosio et al. 2020). Moreover, root tissue N concentration and rates of N-uptake decrease with increasing root length, area, and biomass (Hilbert 1990; Raper Jr et al. 1978; Taub and Wang 2008). Thus, as soil nitrogen becomes more available, generally whole-plant nitrogen increases, whole-plant and photosynthetic nitrogen-use efficiency decreases, and plants allocate more resources to root production (i.e., root to shoot ratios increase) (Hilbert 1990). Yet, nitrogen pools within the plant (i.e., leaf and root nitrogen) interplay with one another and their respective carbohydrate pools, and therefore root nitrogen may not precisely track soil nitrogen availability (Chapin et al. 2011; Raper Jr et al. 1978).

Because nitrogen and phosphorus (P) are often limiting macronutrients, examining their ratio and their ratios to carbon can be informative (Elser et al. 2000a; Sterner and Elser 2009). For balanced plant growth in most terrestrial systems, plants absorb ten times as much (mass-based) nitrogen as phosphorus (Aerts and Chapin 1999; Pons et al. 1998). Therefore, the N:P ratios in leaves are a good indicator of which nutrient is limited. In general, mass-based N:P ratios <14 (molar N:P ratios <35.4) signify nitrogen limitation, whereas mass-based N:P ratios >16 (molar ratios <40.0) correspond to phosphorus limitation with colimitation occurring at mass-based ratios between 14 and 16 (molar ratios between 35.4 and 40.0) (Aerts and Chapin 1999; Güsewell 2004). N:P mass-based ratios for terrestrial plants average 12–13 (molar ratios of 26.5–28.7), owing to the ubiquity of nitrogen limitation in plants globally (Güsewell 2004). Interestingly, average foliar phosphorus content is greater in temperate than in tropical trees (averaging 1.4 vs. 0.75 mg g^{-1} using data from 2962 tree leaves in the TRY database Kattge et al. 2020, David Ellsworth, personal communication), yet across biomes a $2/3^{rds}$ scaling relationship between N and P exists (Niklas 2006, Reich et al. 2009b, Wang et al. 2019).

Several other micronutrients, like calcium, magnesium, sulfur and other trace metals are needed in small quantities by plants, but rarely limit their physiological functioning (Aerts and Chapin 1999; Hawkesford et al. 2012) (Table 1). For example, iron is needed for plant growth and photosynthesis, due to its role in facilitating electron transport in light-reactions of leaves (Kramer and Kozlowski 1979). The oxidation state of iron in the soil interacts with decomposing organic matter and soil nutrients to influence soil nutrient availability for uptake through plant roots (Hall and Silver 2013; Silver et al. 2013). The absorption and concentration of the many other trace metals (aluminum, magnesium, manganese, zinc, copper, and molybdenum) in plant tissues behave similarly and interact with one another and the electrochemical conductivity and chemical composition of the soil (Andresen et al. 2018; Kramer and Kozlowski 1979; Marschner 2012; Mengel and Kirkby 2001; Broadley et al. 2012a; Broadley et al. 2012b) (see Table 1). However, an excess of micronutrients can become toxic, and plants use a variety of mechanisms to avoid toxicity, such as exclusion, restriction of transport from the roots, retranslocation from the plant to roots, root exudation, excretion from the leaves, or compartmentalization (Marschner 2012).

Much of the knowledge about how tropical tree tissue elemental concentrations vary with the environment has focused on variation in foliar chemistry, with the majority of studies focusing solely on carbon, nitrogen, and phosphorus (Reich and Oleksyn 2004, Wright et al. 2004, Niklas 2006, but see Asner and Martin 2016). Additional macronutrients, such as potassium, magnesium, and calcium, have essential biological roles within plants (Table 1), and their concentrations in plant tissues interact with their availability in the soil environment to influence plant growth and forest development (Bond 2010; Hawkesford et al. 2012). Several studies have found that the elemental composition of wood follows soil fertility in tropical forests (e.g., Heineman

et al. 2016; Lira-Martins et al. 2019); yet the extent to which root and leaf elemental concentrations vary with soil fertility, and whether they coordinate in their response, has received less attention. Here, we employ an organismal stoichiometric perspective to compare the concentration of fourteen elemental nutrients in root and leaf tissues of a diverse sample of Angiosperms across a local gradient in soil fertility. In particular, we ask the following questions:

1) Do concentrations of elements in plant root and leaf tissues increase with increasing soil fertility?

*If elemental homeostasis is strong in tropical forest saplings, then tissue nutrient concentrations of both roots and leaves should be invariant to differences in soil nutrient concentrations. However, if the strength of elemental homeostasis varies over a broad range of soil conditions, more fertile soils may lead to higher concentrations of tissue nutrients (*Fig. 1*). Moreover, since roots are the entry point for nutrients, we hypothesize that they may track soil variation more closely than leaves.*

2) How do concentrations of nutrients in roots relate to those in leaves?

*Plant strategies may result in a relatively consistent tissue stoichiometry (*i.e.*, homeostasis). However, due to differences in function, tissues may accumulate nutrients at different rates. For instance, nutrients associated with photosynthetic performance (nitrogen, magnesium, phosphorus) should have higher concentrations in leaf than in root tissues, whereas elements with potential toxicity and only secondary physiological function in leaves (zinc, copper, boron) may accumulate at higher concentrations in roots than in leaves. Based on their physiological function in leaves and root tissues, hypotheses for each element are given in* Table 1.

3) Do plant lineages vary in their leaf and root tissue chemistry-soil environment relationships?

*Because of the variation in Angiosperm plant form, especially regarding roots (*Valverde-Barrantes et al. 2017*), the degree of whole-plant elemental homeostasis may vary with plant lineage. Certain families, like the Fabaceae and Magnoliaceae, have been shown to have higher root N concentrations than others (*Valverde-Barrantes et al. 2017*;* Bergmann et al. 2020*). Families with higher concentrations of root N might have higher concentrations of other elements in roots. We did not formulate any specific expectation as to which families might have more homeostatic regulation than others, but instead employed a sampling design that sought to target taxa across a broad range in plant form and ecological life-history strategy (see* Online Resource 2 Table 2*) to test the hypothesis that plant identity has little effect on plant tissue elemental concentration- soil nutrient concentration relationships.*

Materials & methods

Study site

The Jianfengling forest of Hainan Island, China (18°23′–18° 15′N & 108° 36′–109° 05′E) is a montane tropical rain forest (600–1100 m elevation) on lateric and humic yellow soils that are derived from porphyritic granite (Wu 1995). The soils have low fertility and are characterized by slow rates of mineral cycling when compared to other tropical soils, such as Latisols or Ultisols, intermediate levels of mineral leaching, and an exchangeable base content of about 30 mL kg^{-1} with some accumulation of aluminum. The soils support a vegetation community of broadleaf evergreen trees intermixed with palms and Podocarpaceae that reaches a canopy height of 18 m. The average annual rainfall at Jianfengling from 1965 to 1995 was about 2700 mm, and is seasonal, with most of the rainfall occurring between May and October (Zeng 1995).

Tissue collection

Leaf and fine root tissues were collected from juvenile tropical trees with stem diameters of <10 cm at 1.3 m height (hereafter saplings) from May to July of 2017. Three hundred saplings of 50 tropical tree species (6 individuals per species), chosen to broadly represent 13 Angiosperm families (Online Resource 2 Table 2), were sampled from across a 6.6 km gradient (along a road and trails) spanning an area of secondary and primary tropical forest in the Jianfengling forest reserve (Hogan et al. 2020b). The 13 plant families were chosen to target variation in root morphologies (i.e., from thicker, fleshier roots like those of Magnolids to thinner, more-lignified roots like those in the Fagaceae) (Valverde-Barrantes et al. 2017; Maherali 2017). All selected

species were native species to the local flora, and are considered a representative sample of the flora across the 13 targeted families (Online Resource 2 Table 2; Xu et al. 2015). Saplings were sampled in a balanced design along the 6.6 km gradient collecting 3 individuals from each half of the gradient (Hogan et al. 2020b). Three mature, intact leaves in full sun (i.e., those located on the transect edge) and five lateral entire root systems (i.e., those containing the first three root orders of fine-root tissue, McCormack et al. 2015) were harvested from each sapling. The collected root systems ranged from 0.3 to 3.0 mm in diameter and averaged 98 cm in root length (Hogan et al. 2020b). The 6.6 km gradient was representative of the landscape-scale variation in soil texture and fertility for the greater Jianfengling Forest Reserve (Xu et al. 2015; see Hogan et al. 2020b for further details).

Soil collection & analyses

Following tissue collection, we collected ~1 kg of surface soil (0–10 cm soil depth) from the excavated area. Soils were air-dried for several weeks until completely dry and sieved using a 2 mm mesh (#10) sieve. For each sample, we used approximately 300 g of sieved soil for analysis (Guangzhou Xinhua Agricultural Technical Development Limited Company, Guangzhou, Guangdong, China). Soil texture was measured using the international mechanical soil classification standard. Soil pH was measured using a glass electrode in a 2.5:1 water to soil dilution. We measured soil organic matter employing the high temperature, external-heat, potassium dichromate oxidation volumetric method. Total nitrogen content was measured using the Kjeldahl-distillation titration method, and total phosphorus, available potassium (K), and exchangeable sodium, calcium, and magnesium were all measured using an ammonium-acetate extraction, followed by flame atomic absorption spectrophotometry. Sodium-hydroxide melting-flame atomic absorption spectrophotometry was used to measure total soil K. The alkali-solution diffusion method was used to measure available (i.e., alkali-hydrolysable) soil N. Soil available P was measured using by the hydrochloric acid–ammonium fluoride extraction and the molybdenum antimony anti-coloring method, and soil base saturation and cation exchange capacity were measured with the ammonium acetate methods.

Tissue homogenization and elemental analysis

Root tissue samples were washed thoroughly, removing soil, and both surfaces of leaf samples were wiped with a paper towel. Leaf and root tissues were oven-dried at 70 °C for at least 48 h until completely dry. Tissue samples were placed individually into sterile 5 mL propeleyne screwcap vials and finely ground using a Fisher Beadmill 24 multi-sample homogenizer (Fisher Scientific, USA) with 5 mm stainless steel beads over multiple 30-s cycles at high speed. Prior to homogenization, stainless steel beads were cleaned and sterilized with ethanol to prevent contamination. This method is commonly used and does not result in systematic bias in tissue trace metal or other elemental concentrations (Maia and Shaddox 2019). Homogenized leaf and root tissues were microweighed (2–3 μg) into aluminum micro tins on a Mettler Toledo XS3DU microbalance (Metler Toledo, Columbus, OH, USA) and put through continuous flow isotope ratio mass spectrometry and elemental analysis using a Thermo Delta Plus GC-IRMS (Thermo-Fisher, Waltham, MA, USA) for analysis of carbon and nitrogen. Agricultural macronutrient analyses were done using wet-acid digestion (following the methods in Jones Jr 2001) at the Soil Testing and Plant Analysis Lab at Louisiana State University. Briefly, one-half (0.5) grams of finely-ground plant tissue was digested in 2.2 mL of deionized water using 5 mL of concentrated Nitric Acid. The solution was heated for 2.75 h at 125 °C. At the end of the heating, 3 mL hydrogen peroxide was added, and the solution was cooled and filled to a volume of 20 mL with deionized water. Elemental concentrations of aluminum (Al), boron (B), calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), sodium (Na), phosphorus (P), potassium (K), sulfur (S), and zinc (Zn) of digested tissue samples were determined using inductively coupled plasma optical emission spectroscopy (Avio 500 ICP-OES, Perkin Elmer, Waltham, MA, USA). Tissue concentrations of molybdenum were mostly below the detectable range, so we did not include them in our analyses.

Statistical analyses

A principal components analysis (PCA) was used to identify variables that represent the main axes of variation in soil nutrients. Data were scaled and centered before the PCA ordination. The PCA showed that the

first two axes explained 61.8% of the variance. Three soil variables: base saturation (soil BS), total nitrogen (soil N), and total phosphorus (soil P) mostly characterized the soil differences among plant habitats (Online Resource 2 Fig. 5). Variation in those three soil parameters encompasses the range of soil variability among habitats, and they were only weakly correlated with one another (Pearson's r values all ≤0.38, Online Resource 2 Fig. 6). Therefore, we used these three soil variables as representations of soil fertility.

To address the research question of whether tissue nutrient concentrations track soil fertility (i.e., question 1), we used linear mixed-effects models (LMMs). Tissue nutrient concentration data were $\log_{10}$-transformed to improve the normal distribution of error. We determined that random intercept terms for both species and family were justifiable given that we had a balanced sampling design of six individuals per species for 50 species and were interested in differences among species and by plant lineage (i.e., family, question 3). LMMs were fit separately for each of the fourteen tissue nutrients using the 'lme4' package (Bates et al. 2015) in R v.3.6.0 (R Core Team 2019). A saturated model was built including random intercepts for species and family and fixed effects for soil N, soil P, and soil BS, and their interactions with organ type (i.e., leaf vs. root): $\log_{10}([Tissue\ Nutrient]) = (\beta soilN + \beta soilP + \beta soilBS) * organ + 1 \mid family + 1 \mid species$. Model selection was then performed using the 'step()' function from the 'lmerTest' package (Kuznetsova et al. 2017) in R v.3.6.0 (R Core Team 2019), which implements backward elimination of random-effect terms where applicable, followed by backward elimination of fixed effects based on model Akaike Information Criterion. Final models were fit using residualized maximum likelihood estimates, which accounts for the number of model parameters when estimating parameter values by applying the likelihood function over the least-squares residuals. Statistical significance was determined using the Wald method.

To analyze if leaf nutrients directly tracked root nutrients (i.e., passive transport of nutrients among plant organs, question 2), we used a paired Wilcoxon signed-rank test (Wilcoxon 1945). It is a nonparametric statistical test that compares whether the population mean ranks differ, where the null hypothesis is that the difference between pairs follows a symmetric distribution around zero. It is often used as a nonparametric alternative to a paired Student's T test to test if two samples come from populations that have the same distribution, because it does conform to the same statistical assumptions of normality as a paired Student's T test. In the context of our data, a non-statistically significant result means that the ranks of tissue element concentrations between leaves and roots of individual saplings do not differ, and can thus be considered equal. On the other hand, if the test statistic is significant at the .05 level of statiscial significance, we interpret this to mean that concentrations of elements between leaves and roots of individual saplings are not equal.

Results

We first discuss the measured stoichiometry of tissues in context. Then, we present results from the linear mixed-effects models for each of the 14 elements analyzed. Finally, we summarize results related to our three research questions.

Carbon, nitrogen and phosphorus stoichiometric ratios

Average (± standard error) molar C:N ratios were 44.8 ± 1.1 (range: 13–132) for roots, and 34.7 ± 0.8 (range: 11–107) for leaves (Fig. 2a); mass-based C:N ratios averaged 38.5 ± 1.0 (range: 10.8–113.2) for roots and 29.7 ± 0.7 (range: 9.8–92.0) for leaves. Molar ratios for N:P were 92. 8 ± 2.4 (range: 13–434) for roots and 80.4 ± 1.2 (range:21–174) for leaves (Fig. 2b); mass-based N:P ratios averaged 43.3 ± 1.3 (range: 5.9–196.4) for roots and 37.2 ± 0.08 (range: 9.6–116.8) for leaves. Lastly, molar ratios for C:P were 3869.4 ± 103.1 (range:600–10,972) for roots and 2739.6 ± 64.6 (range: 395–6230) for leaves (Fig. 2c); mass-based C:P ratios averaged 1500.2 ± 39.7 (range: 223.4–4254.5) for roots and 1060.3 ± 25.1 (range: 153.1–2510.5) for leaves. Horizontal lines in Fig. 2 show global nutrient ratio averages as reported by Sterner and Elser (2009)). The high C:P and N:P ratios relative to the global averages for terrestrial plants show that, generally, tissues were phosphorus-poor (Fig. 2).

Tissue nutrients in relation to soil nutrients – Results from LMMs

Of the fourteen tissue nutrients analyzed, half showed increasing concentrations with increasing soil fertility; those being nitrogen (Fig. 3b), boron (Fig. 3c), phosphorus (Fig. 3g), potassium (Fig. 3i), manganese (Fig. 3k),

copper (Fig. 3m), and zinc (Fig. 3n). Concentrations of three of fourteen tissue nutrients: magnesium (Fig. 3e), sulfur (Fig. 3h), and calcium (Fig. 3j) showed no relationship to soil fertility. Tissue concentrations of carbon (Fig. 3a) and aluminum (Fig. 3f) showed a slightly decreasing trend with soil fertility. Two of fourteen tissue nutrients showed divergent relationships between roots and leaves (i.e. non-parallel slopes or strong interactions of soil fertility and leaf type). With increasing soil P, sodium content in leaf tissues showed a decreasing trend but increased slightly in roots (Fig. 3d). Conversely, with increasing soil P, iron concentrations in roots decreased somewhat, but iron concentrations in leaves were invariant (Fig. 3l).

Tissue carbon concentration responded weakly and positively to soil N (β coefficient of 0.01), more-strongly negatively to soil P (β of −0.26), and did not vary with soil BS. Leaf carbon concentrations were statsistically lower than root carbon concentrations (Fig. 3a, β of −0.03). There was a minimal degree of interfamilial variation in tissue C concentrations, with species in the Lauraceae, Sapotaceae, Anacardiaceae, Pentaphylacaceae, Sapindaceae, Juglandaceae, and Fagaceae having slightly higher tissue carbon concentrations than those in the Theaceae, Ebenaceae, Burseraceae, Rutaceae, Moraceae, Annonaceae and Magnoliaceae (Online Resource 1 S1).

Tissue nitrogen concentration did not respond to soil N; that is, the best-fitting LMM for tissue nitrogen did not include soil N as an explanatory variable but included soil P instead. Soil P had a relatively-strong positive effect on tissue N (β of 0.49, Fig. 3b). Leaf tissue N concentrations were higher than root N concentrations (β of 0.05), and there was a weak interaction between organ type and soil BS (β negligible), with increasing soil BS slightly decreasing leaf N. Interfamilial variation in tissue N was greater than that of tissue C. The Rutaceae and Lauraceae had significantly more N in tissues. The Pentaphylacaceae, Fagaceae and Theaceae had significantly less N in tissues than the remaining 9 Angiosperm families, whose random effect confidence intervals included zero (Online Resource 1 S2).

Soil P had a strong effect on tissue phosphorus (β of 1.71, Fig. 3g), soil N had a minute negative effect on tissue phosphorus (β of −0.07), and soil BS had little effect on tissue phosphorus (β negligible). Tissue phosphorus concentration was greater in leaves than in roots (β of 0.12). Among families, species in Rutaceae had slightly greater tissue phosphorus concentrations, and species in the Fagaceae, Pentaphylacaceae, and Theaceae had slightly less tissue phosphorus than the remaining 11 Angiosperm families (Online Resource 1 S7).

Boron, potassium, manganese, copper, and zinc concentrations tended to increase in tissues with soil nutrient availability. In the case of boron (Online Resource 1 S3), soil P was removed as an explanatory variable during model selection. Soil N had a weak, but statistically insignificant, positive effect on tissue boron concentrations (β of 0.03), which were greater in leaves than roots (β of 0.47, Fig. 3c). Species in the Moraceae had greater than average tissue boron concentrations, while Lauraceae species had less than average tissue boron concentrations. Tissue potassium concentrations responded positively to soil P (β of 0.95, Fig. 3i), negatively to soil N (β of −0.07), and were unaffected by soil BS (β negligible). Potassium was greater in leaf than in root tissues (β of 0.11, Fig. 3i), with species in the Rutaceae, and Moraceae having significantly greater potassium concentrations and species in the Pentaphylacaceae, Theaceae and Fagaceae having significantly lower concentrations than the familial average (Online Resource 1, S9). Concerning manganese concentrations in tissues, soil P had a relatively-strong positive effect (β of 1.72, Fig. 3k), and soil N had a weak negative effect (β of −0.19). Additionally, manganese concentrations were greater in leaves than they were in roots (β of 0.39, Fig. 3K). Considerable intraspecific variation existed in leaf and root manganese concentrations, however all families were statistically equal except for the Moraceae which had greater magnesium concentrations than the other 13 plant families (Online Resource 1, S11). Copper concentrations in plant tissues responded positively to increasing soil P (β of 0.90, Fig. 3m), and weakly negatively to soil N (β of −0.09). Soil BS had no strong effect on tissue copper concentrations (β of 0), but was included in the best-fitting model. Contrary to many of the other nutrients, copper concentration was greater in root tissues than leaf tissues (β of −0.34 for leaf type). Additionally, copper concentrations were higher in the Rutaceae and Lauraceae, and lower in the Theaceae and Pentaphylacaceae than in the other 10 plant families (Online Resource 1, S13). Lastly, zinc concentration was also greater in roots than in leaves (β of −0.48 for leaf type, Fig. 3n). Increasing soil N led to a decrease in leaf zinc concentration (interaction-term β of −0.13). Lauraceae and Rutaceae had statistically

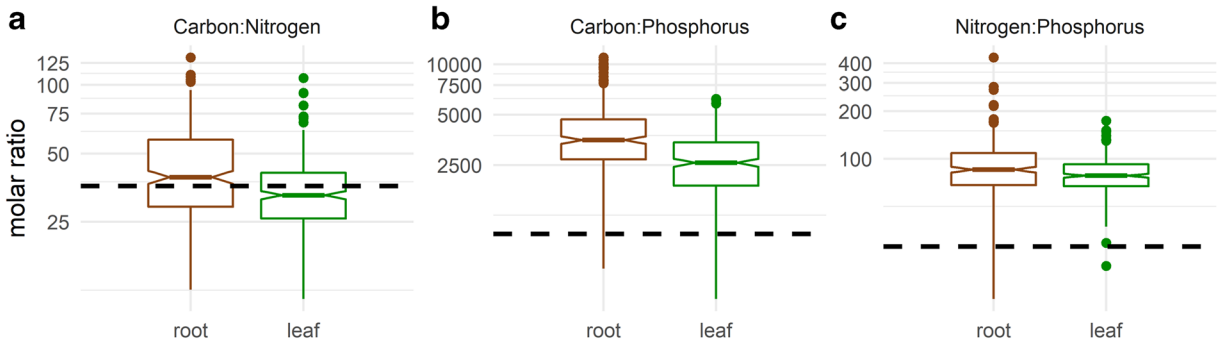

Fig. 2 Box and whisker plots for foliar and root elemental molar ratios for C, N, and P for 300 individuals sampled in the Jianfengling Forest Reserve, China. Vertical axes are log-scale. Boxplots show means (center line), interquartile ranges (boxes), and the smallest and largest values within 1.5 times the interquartile range (whiskers). Points are >1.5 times outside the interquartile ranges. Horizontal dashed lines represent the global average of foliar C:N, C:P, and N:P molar ratios, as reported by Sterner and Elser (2009) (36, 968, and 28, respectively)

greater than average amounts of tissue zinc, and Theaceae and Fagaceae had statistically less than average amounts of tissue zinc among all plant families (Online Resource 1, S14).

Concentrations of elements in plant tissues with soil fertility

Three nutrients showed no relationships with soil nutrient availability, those being magnesium, sulfur, and calcium. Soil N had a slight positive effect on tissue magnesium concentration (β of 0.06); however, it led to a decrease in leaf magnesium (interaction-term β of −0.07). Amounts of magnesium were found to be higher in leaf than roots tissues (β of 0.23). Species in the Lauraceae, Sapotaceae, and Fagaceae had significantly lower than familial average trends, and species in the Moraceae had significantly higher than familial average trends in tissue magnesium concentrations (Online Resource 1, S5). The best-fitting model for sulfur included a fixed effects for soil BS (β negligable) and organ type (β of 0.03 for leaves) and random intercept terms for family and species, thus we can understand plant sulfur concentration to be invariant with changes in soil nutrients (Fig. 3h). Again, species in the Lauraceae and Rutaceae had greater than average amounts of tissue sulfur, while those from the Fagaceae had less (Online Resource 1 S8). A slight negative effect of soil N (β of −0.06) and negligible positive effect (β of 0.01) of soil BS on tissue calcium emerged from the LMM fit for calcium (Online Resource 1, S10). However, calcium concentrations of tissue were invariable with environmental variation in soil P (Fig. 3j). Calcium concentrations were greater in leaves than in roots (β of 0.40 for leaves). Plants in the Sapotaceae and Fagaceae families had lower than average calcium concentrations.

Finally, two plant nutrients, sodium, and iron exhibited diverging trends among roots and leaves. For sodium tissue concentrations, soil N had a positive effect (β of 0.06) and soil P had a non-significant positive effect (β of 1.01, but with confidence interval overlapping zero). Sodium concentrations were higher in leaves than in roots (β of 0.23), and both increases in soil P (interaction term β of −3.89) and soil BS (interaction term β of −0.01) caused decreases in leaf sodium concentration (Fig. 3d, Online Resource 1, S4). Amounts of iron in root tissues decreased with increasing soil P (β of −0.94), and iron was higher in root tissues than in leaf tissues (β of −1.67 for organ type leaf). Soil N interacted to increase leaf iron concentrations (β of 0.11), while soil BS had a negative effect (β of −0.01), hence the slight difference in slopes in Fig. 3l (Online Resource 1, S12).

Comparing element concentrations in roots and leaves

We found significant differences in the ranks of tissue nutrient concentrations between plant organs. Wilcoxon probabilities were highly statistically significant (all p values << 0.001) for carbon (Fig. 4a), nitrogen (Fig. 4b), sodium (Fig. 4d), magnesium (Fig. 4e), phosphorus (Fig. 4g), potassium (Fig. 4i), calcium (Fig. 4j), manganese (Fig. 4k), iron (Fig. 4l), zinc (Fig. 4n). For those nutrients, ranks of leaf and root nutrient concentrations were not symmetric, in that when the concentration of an

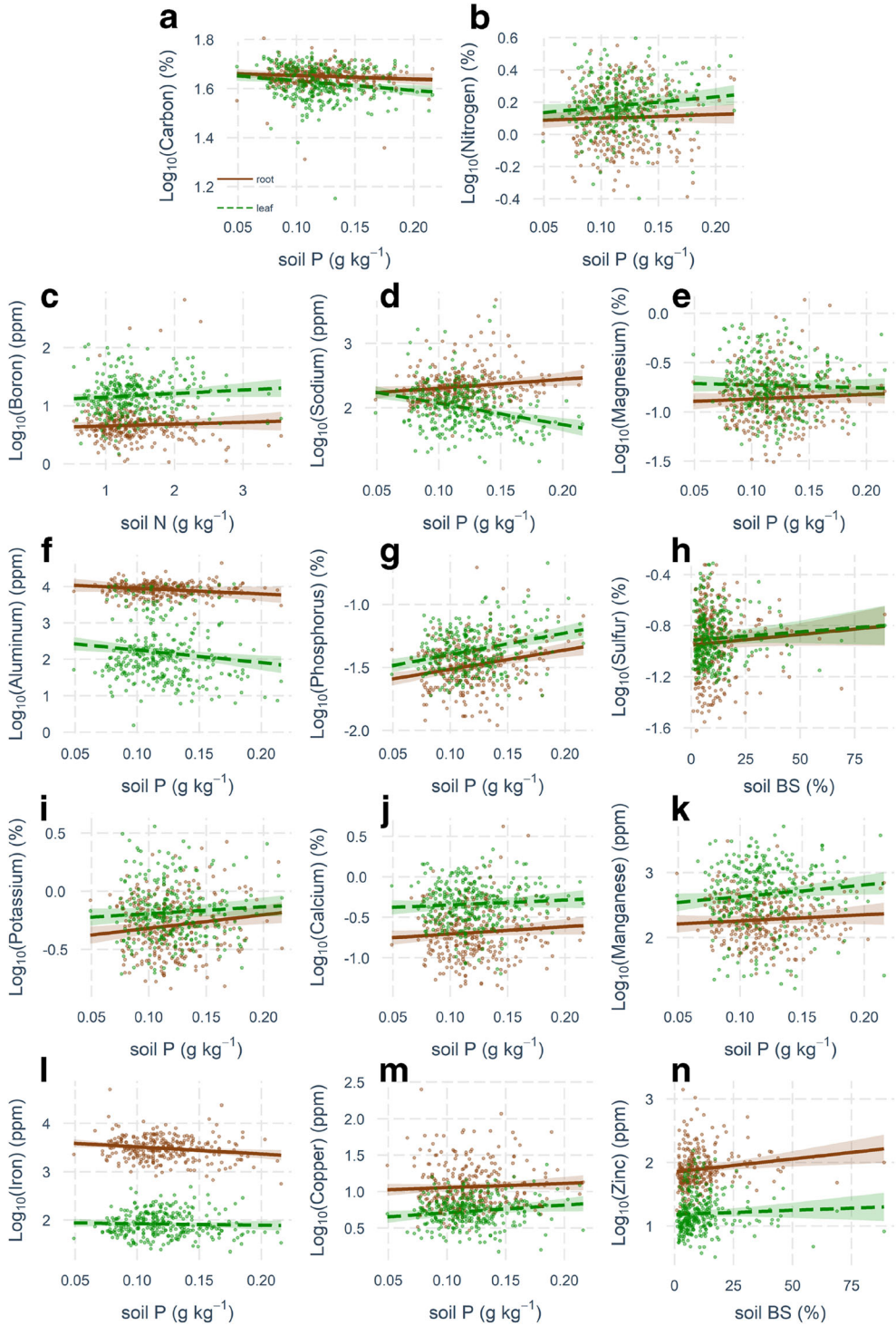

Fig. 3 Plant tissue Carbon **a**, Nitrogen **b**, Boron **c**, Sodium **d**, Magnesium **e**, Aluminum **f**, Phosphorus **g**, Sulfur **h**, Potassium **i**, Calcium **j**, Manganese **k**, Iron **l**, Copper **m** and Zinc **n** in relation soil fertility (i.e., either soil nitrogen – soil N, phosphorus – soil P, or base saturation – soil BS). Measured concentrations of tissue nutrients (roots in brown and leaves in green) and linear mixed model fits (with 95% confidence intervals) are plotted. The soil fertility variable on the x-axis varies based on the best fitting linear mixed-effects models, fitted after backward selection of variables (see methods). For a complete statistical description of linear mixed-effects models, see Online Resource 1

element was relatively high in root tissue, it tended to be low in leaf tissue, and vice versa. Ranks of tissue nutrient concentrations were not significantly different for boron (Fig. 4c), aluminum (Fig. 4f), sulfur (Fig. 4h), and Copper (Fig. 4m).

Variation in relationships due to plant family and species

Random effect variance of fitted LMMs, attributed to family and species identity, was low ranging from <0.1 for carbon to 0.21 for aluminum, and averaging 0.06 (values are $\log_{10}$-transformed tissue concentration units). Thus, in all LMMs, the random effects of species and family accounted for a small amount of variation about model fits, although in all cases where models included random effects for families and species, the amount variation explained by families was slightly greater than the variation explained by species (i.e., $\tau_{family} > \tau_{species}$). Intraclass correlations (ICC), or the proportion of variation explained by the random effect grouping structure of the model (i.e., between species and family groupings), ranged from 0.10 for iron to 0.52 for magnesium, averaging 0.34 across all 14 models (Online Resource 1), signifying that tissue nutrient measurements within random effects groupings were weakly to moderately related (note that ICC values can be interpreted like Pearson correlation coefficients). Yet, in some instances, statistically significant deviations from model averages were observed for plant families, and to a lesser degree, for individual species (see caterpillar plots in Online Resource 1).

Discussion

Contextualizing the nutrient concentration of tissues from Jianfengling saplings

Tropical forests such as Jianfengling are typically P-limited systems (Vitousek 1984; Vitousek and Sanford Jr 1986), therefore we first discuss root and leaf tissue nutrient concentrations from Jianfengling in relation to global averages and other forests in China. First, Jackson et al. (1997) reported global nutrient pools for fine roots to be 488 ± 9.8 mg g^{-1} carbon, 11.7 ± 0.7 mg g^{-1} nitrogen, 1.1 ± 0.2 mg g^{-1} phosphorus, 3.0 ± 0.6 mg g^{-1} potassium, 4.1 ± 1.0 mg g^{-1} calcium, 1.4 ± 0.3 mg g^{-1} magnesium, and 0.9 ± 0.1 mg g^{-1} sulfur, with living roots containing more nutrients than dead roots (Gordon and Jackson 2000). Second, in a meta-analysis of root decomposition rates by Silver and Miya (2001), the initial nutrient concentration of fine roots (those <2 mm in diameter) was 0.94 ± 0.05 mg g^{-1} nitrogen, 0.5 ± 0.1 mg g^{-1} phosphorus, 2.3 ± 0.4 mg g^{-1} calcium, with mass-based C:N ratios averaging 67 ± 6. In Jianfengling, we recorded nutrient concentrations of 449 ± 24 mg g^{-1} carbon, 13.9 ± 0.3 mg g^{-1} nitrogen, $3.6 \pm <0.1$ mg g^{-1} phosphorus, 5.9 ± 0.2 mg g^{-1} potassium, 2.8 ± 0.2 mg g^{-1} calcium, $1.7 \pm <0.1$ mg g^{-1} magnesium, and $1.4 \pm <0.1$ mg g^{-1} sulfur in surface entire root systems for 300 juvenile trees (Figs. 3 & 4). Thus, tissue nutrient concentrations in the juvenile trees of Jianfengling are similar in carbon and nitrogen to values reported in the literature, but much lower than reported values for phosphorus. The other macronutrients (i.e., potassium, calcium, magnesium, and sulfur) were comparable to reported values.

Concentrations of other elements, like cations and metals, were lower than have been reported in the literature. Specifically, Zhang et al. (2012) summarized leaf tissue nutrient data from 702 plant species (of 66 families) from 91 sites across China. Data from this study find higher aluminum (1.03 ± 0.14 vs. 0.83 ± 0.07 mg g^{-1}), and manganese (0.76 ± 0.04 vs. 0.23 ± 0.02 mg g^{-1}) concentrations in leaves than reported by Zhang et al. (2012), but lower sodium (0.16 ± 0.02 vs. 8.91 ± 0.94 mg g^{-1}), iron (0.10 ± 0.01 vs. 0.57 ± 0.06 mg g^{-1}), calcium (5.49 ± 0.19 vs. 15.5 ± 0.57 mg g^{-1}), sulfur (1.40 ± 0.04 vs. 3.26 ± 0.23 mg g^{-1}), potassium (7.76 ± 0.30 vs. 13.05 ± 0.42 mg g^{-1}), phosphorus (0.47 ± 0.01 vs. 1.41 ± 0.05 mg g^{-1}), and nitrogen (16.13 ± 0.33 vs. 19.96 ± 0.43 mg g^{-1}). One explanation for these differences is that soils at Jianfengling, Hainan Island China are more-highly weathered than most of the soils on mainland China, receiving substantially more rainfall than most parts of China. High levels of aluminum and manganese in the tissue of saplings from Jianfengling point to acidic soil conditions with low amounts of available nutrients (Wu 1995; Xu et al. 2015),which are typical of tropical montane forests with weathered soils.

On tissue carbon concentration, Ma et al. (2018) report in a global meta-analysis (containing >20,000 measurements) that leaf C concentration is equal to or greater than root C concentration, measuring 470 ± 40 mg g^{-1} and 460 ± 50 mg g^{-1}, respectively. We found leaf C concentration to measure, on average, over 2%

lower than in root C concentration (426 ± 40 mg g^{-1} for leaves and 449 ± 20 mg g^{-1} C for roots). Although these differences are not large, they are statistical significant at the individual level (Wilcoxyn sign ranked test *p value* highly significant, Fig. 4a), and across the 50 species we sampled (Online Resource 1, Fig. 3a). One explanation for this difference could be plant ontogeny; we were working with saplings, which tend to be photosynthate-limited due to their understory habitats and relatively small total leaf areas, which could create a greater

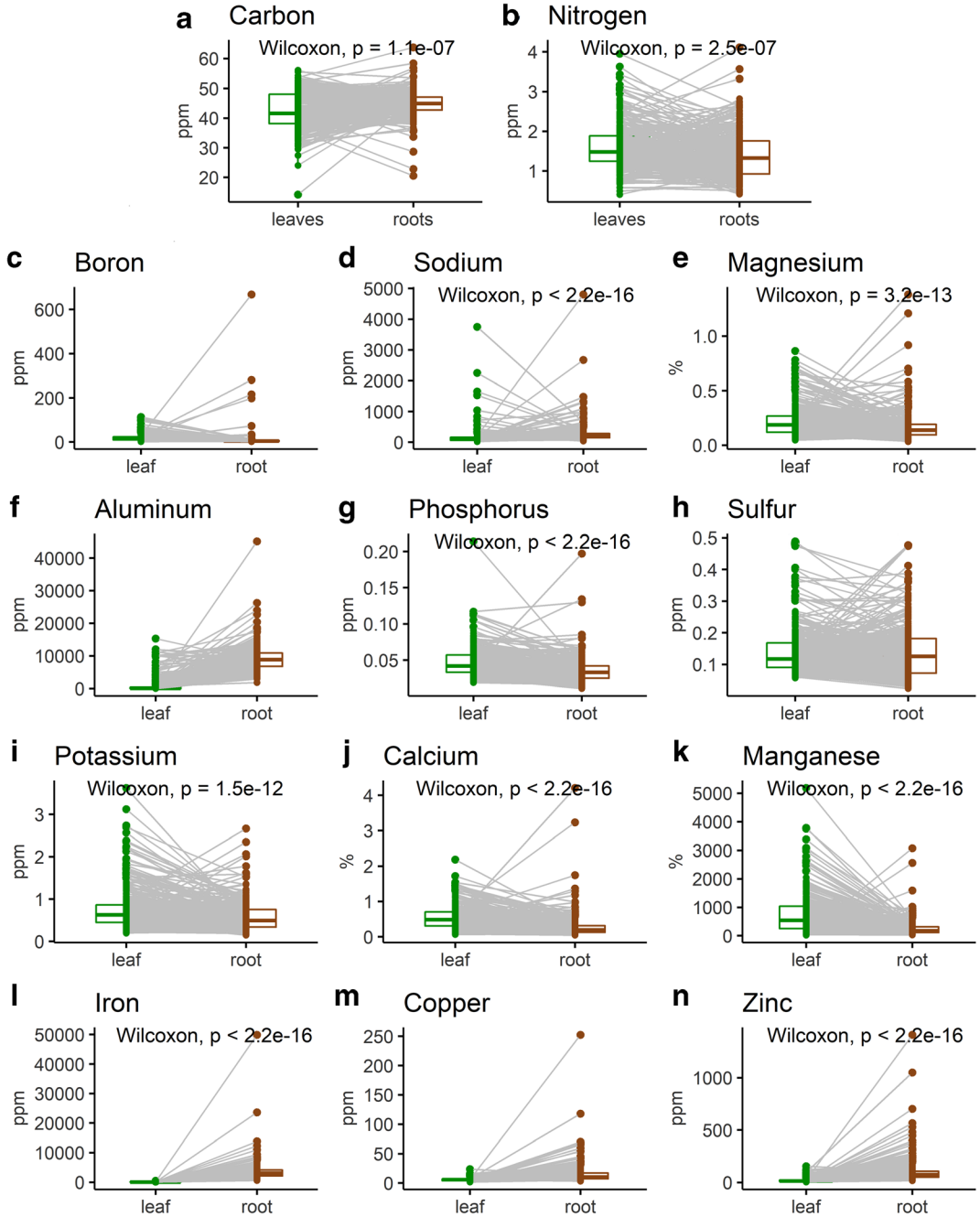

Fig. 4 Comparison of leaf and root concentrations of 14 essential elements. Boxplots show averages and interquartile ranges. Paired samples are connected with grey lines, and statistically significant paired-Wilcoxon signed-rank test probabilities are shown

difference in tissue carbon concentrations of leaves and roots (i.e., a greater degree of whole-plant carbon limitation). Secondly, differences could exist because of variation in relative amounts of the common C-containing molecules, such as lignin, cellulose, sugars, proteins, and lipids, of the leaf and root tissues of saplings relative to adult trees (Hobbie and Werner 2004). Indeed, Martin et al. (2013) found that stem wood of tropical saplings in Panama had 21% less hemicellulose and 36% more lignin, making them about 2% greater in carbon content than conspecific adult tress. Additionally, differences in plant laible carbon allocation varies with tree age, with younger trees allocating relatively more carbon belowground than their established adult, non-light limited counterparts (Chapin et al. 1990; Dietze et al. 2014; Kozlowski 1992; Raich et al. 2014).

Do concentrations of elements in plant root, and leaf tissue track increases soil fertility?

Our first research question asked whether root and leaf tissue elemental concentrations increase with increasing soil fertility. Concentrations of N and P in both root and leaf tissue increased with more soil P, but tissue C concentration showed a slight decreasing trend (Fig. 3a). Eight of the other eleven elements we studied (excluding C, N, and P) showed relationships with soil fertility. The three nutrients that did not display links to soil fertility were boron, aluminum, and sulfur, which have either unclear or substitutable functions within the plant (Table 1) and may be toxic at high levels. The eight nutrients that responded to some degree to variation in soil nutrient concentrations were sodium, magnesium, potassium, calcium, manganese, iron, copper and zinc (Fig. 3), which are all cations with unique, indispensable functions within the plant cell (Table 1).

Literature-based hypotheses for changes in tissue nutrient concentrations to an increase in soil fertility (Table 1) were correct for half (7 of 14) of the elements we studied. For nitrogen, phosphorus, and potassium, hypothesized increases in tissue nutrient concentrations with increasing soil fertility were supported by the data (Fig. 3b, g, i). For carbon and aluminum, the hypotheses of decreasing tissue concentrations with increasing soil fertility were confirmed (Fig. 3a, f), and additionally, for sulfur, iron, and copper we were able to confirm the literature-based invariant relationships in tissue nutrient concentrations to variation in soil nutrients (Table 1). On the other hand, we observed no change in tissue concentrations of magnesium, where the literature predicted an increase (Fig. 3e), and we found a decrease in leaf sodium with increasing soil P in opposition to the hypothesized increase, although concentrations in root tissues did increase (Fig. 3d). We observed a slight increase in tissue zinc concentrations with increasing soil nutrient concentrations (Fig. 3n), despite the literature supporting the hypothesis that concentrations should decrease. However this trend was driven by only a few datapoints for plants collected in soils with high soil BS. Similarly, we observed very small increases in tissue boron and manganese concentrations with soil N and P, respectively (Fig. 3c, k), where the literature supported that relationships should be invariant.

Furthermore, responses of tissue nutrients to soil fertility did not vary systematically with Marschner's 4-group nutrient classification (Marschner 2012; Kirkby 2012; Mengel and Kirkby 2001). Recall that group 1 nutrients are taken up as ions, group 2 nutrients are absorbed as inorganic anions or acids, group 3 nutrients are mostly taken up as cationic compounds (excepting chlorine which is group 3 anion), and group 4 nutrients are actively taken up in their ionic forms. At least one element from all four groups (nitrogen – group 1, boron and phosphorus – group 2, potassium and manganese – group 3, and copper and zinc – group 4) increased in concentration in plant tissues with increasing soil fertility. Carbon (a group 1 nutrient) and aluminum (a group 4 nutrient) were the two that decreased slightly with increasing soil fertility. Those showing no change were magnesium, calcium (both group 3 nutrients) and sulfur (a group 1 nutrient). Sodium (a group 3 nutrient) and iron (a group 4 nutrient) interacted with soil fertility to show diverging trends between root and leaf tissue concentrations with increasing soil fertility.

Tissue phosphorus showed the strongest increasing trend with soil P availability (Fig. 3e). This suggests that plants in Jianfengling are P-limited (Fig. 2, Vitousek 1984; Vitousek and Sanford Jr 1986) and that adding P to the soil would have the greatest effect on plant growth and likely increase tissue nutrient concentrations. Indeed, SJ Wright (2019) reported a strong effect of P-addition on leaf tissue P concentration (Hedges g effect size of 1.7) in a meta-analysis of nutrient addition experiments in tropical forests. However, responses of tropical tree tissue stoichiomety to increases in P probably varies with species sensitivities (Turner et al. 2018), tree size (Wright et al. 2018), and by plant organ

(e.g., roots, stems and leaves). Wurzburger and Wright (2015) reported clear increases in fine root tissue P concentration when adding P in a lowland Panamanian forest, with roots decreasing in morphological traits associated with nutrient acquisitiveness per the "do it yourself" root functional strategy (*sensu* Bergmann et al. 2020); that is, roots had less total biomass, decreased in root length, but increased in specific root length at high soil P concentrations. At Jianfengling, we found similar morphological shifts in the root systems from which tissue nutrients were measured, where diameter was narrower, and specific root length (SRL) and root system branching intensity were greater in lower soil fertility areas than in areas with more soil nutrients; increased concentrations of soil bases and P were significantly related to increased SRL (Hogan et al. 2020b).

Tissue nitrogen showed a similar pattern to phosphorus, increasing with soil P availability, but to a lesser degree (Fig. 3). Soil N was not backward selected as a fixed effect in the model selection process for the LMM for tissue N concentrations, suggesting that saplings are more limited by soil P than by soil N. However, Wurzburger and Wright (2015) reported no change in root tissue N with nutrient addition in a lowland Panamanian forest. The variety of strategies by which, and sources from which, plants obtain N, along with the demand for and homeostatic movement of N within the plant, make the interpretability of root N concentration, by itself, difficult, although some progress on conceptualizing root N in relation to root economis is being made (McCormack and Iversen 2019; Bergmann et al. 2020). Ultimately N concentrations in leaf and root tissues depends on a suite of abiotic (e.g., diffuse nutrient flows in the soil) and biotic biogeochemical processes (e.g., microbial and fungal nutrient mobilization in the soil), which interact with plant and soil stoichiometry (Aerts and Chapin 1999; Brown 1978; Elser et al. 2000b; Gordon and Jackson 2000; Güsewell 2004; Marschner 2012).

How do concentrations of nutrients in roots relate to those in leaves?

We found that concentrations of carbon, sodium, iron, copper, and zinc were greater in roots than leaves (Fig. 3), whereas concentrations of nitrogen, boron, magnesium, phosphorus, sulfur, potassium, calcium and manganese were greater in leaves than in roots. Elements that had higher concentrations in leaves than in roots, excluding magnesium, calcium, and sulfur, tended to increase in concentration in leaves with increasing levels of soil nutrients. This supports the idea that, at least for some elements, there is within-plant homeostatic elemental regulation. The most supportive evidence we found for homeostatic regulation came from the results of the Wilcoxon sign-ranked test, which was significant for carbon, nitrogen, phosphorus and seven of the eight, above mentioned elements that responded to variation in soil nutrient concentration. The evidence that when element concentrations are high in roots, they tend to be lower in leaves, and vice versa, points to selective plant use, movement and storage of these elements within plant tissues and cells. Copper was the lone case of an element that responded to soil nutrient availability, but was not regulated within the plant. Active within-plant elemental regulation may be responsible for these patterns, as we have discussed, however alternative mechanisms could result in similar patterns. First, roots can act as a filter for certain elements (e.g., boron, aluminum, iron, copper and zinc), excluding their movment into plants (Marschner 2012), actively uptaking other less toxic cations in their place (i.e. selecting against their uptake, Kahle 1993) or though complex alterations of soil biogeochemistry and pH via root exudates (Jones and Darrah 1994). Moreover, certain elements may accumulate in leaves (e.g., calcium, if they are in compounds that are phloem-insoluble or too large for phloem transport) (Hill 1980).

Do plant lineages vary in their leaf and root tissue chemistry-soil environment relationships?

Among all elemental concentrations in tissues, there was considerable variation within families and species. In other words, among plant variation far exceeded any intraspecific or intrafamilial variation (σ^2 for random effects <0.1, except for aluminum where σ_2 was 0.21, Supplement 1). Yet, for each element, certain families differed statistically from the fitted trend for all families. There was no interfamilial or intraspecific variation (i.e., no statistically significant effects for levels of random family or species intercept terms) in modeled tissue carbon concentration; however, there was for each of the 13 other elements studied (see caterpillar plots in Online Resource 1). For example, plants in the Theaceae had higher tissue aluminum concentrations than the modeled trend (Online Resource 1, S6) Thus, in certain cases, plant lineage does modulate the relationship

strength between leaf and root tissue chemistry and the soil environment, which is already subtle at the local environment and individual plant scales. This is likely due to slight differences in the physiological functioning among plant lineages, which are the result of their evolved biological differences and variation in life-history strategies. Plant evolutionary history has strongly influenced root morphologies (Valverde-Barrantes et al. 2017), which likely has direct effects on nutrient uptake physiologies, soil habitat preferences, or mechanisms for dealing with soil element toxicitiy (Marschner 2012). However, with an incomplete sampling of the Angiosperm phylogeny, it is difficult to thoroughly assess the role of plant lineage, as deeper nodes in the phylogeny probably drive patterns of nutrient concentration variation among taxa (Kerkhoff et al. 2006). For example, variation for some nutrients, especially these involved in cell wall structures (carbon, calcium, magnesium) occurs among plant orders, rather than families or species (Broadley et al. 2004). In our case, any slight differences in physiology (e.g., nutrient requirement or use) seem to be overshadowed by larger nutrient constraints of the soil environment itself. This result is congruent with previous research that has found a wide range in tropical tree tissue nutrient concentrations (Hattenschwiler et al. 2008; Townsend et al. 2007) and their stoichiometries (Townsend et al. 2008; Elser et al. 2010).

In summary, using data on root and leaf tissues of 300 saplings of 50 species from across a representative range of local habitat variability in soils of a tropical montane forest, we found stable tissue concentrations for half of the 14 elements we considered. Therefore, at least some-degree of elemental homeostasis, that is, the maintenance of constant levels of tissue nutrients despite changes in the nutrient concentration of the external soil environment, was observed. In cases where plant tissue nutrients either decreased or increased with soil nutrient levels, trends tended to be weak, potentially because of nutrient limitation in highly leached tropical forest soils. Thus, the physiological functioning of saplings in the montane tropical forest at Jianfengling, China is stoichiometrically constrained across environmental variation (i.e., has a broad stoichiometric knife-edge, at least with respect to the range of soil environments studied here). However, their stoichiometry does respond to variation in environment and resource quantity with potential for nutrient regulation between plant organs (i.e., roots and leaves), especially for elements that have an critical physiological functions in leaves (e.g., nitrogen, phosphorus, potassium, magnesium, and manganese).

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Data availability Data used in this study are available for download at: https://doi.org/10.6084/m9.figshare.8283593.v2 (Hogan et al. 2020a)

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