Mineral Elements of Subtropical Tree Seedlings in Response to Elevated Carbon Dioxide and Nitrogen Addition

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Abstract

Mineral elements in plants have been strongly affected by increased atmospheric carbon dioxide (CO₂) concentrations and nitrogen (N) deposition due to human activities. However, such understanding is largely limited to N and phosphorus in grassland. Using open-top chambers, we examined the concentrations of potassium (K), calcium (Ca), magnesium (Mg), aluminum (Al), copper (Cu) and manganese (Mn) in the leaves and roots of the seedlings of five subtropical tree species in response to elevated CO₂ (ca. 700 μmol CO₂ mol⁻¹) and N addition (100 kg N ha⁻¹ yr⁻¹) from 2005 to 2009. These mineral elements in the roots responded more strongly to elevated CO₂ and N addition than those in the leaves. Elevated CO₂ did not consistently decrease the concentrations of plant mineral elements, with increases in K, Al, Cu and Mn in some tree species. N addition decreased K and had no influence on Cu in the five tree species. Given the shifts in plant mineral elements, Schima superba and Castanopsis hystrix were less responsive to elevated CO₂ and N addition alone, respectively. Our results indicate that plant stoichiometry would be altered by increasing CO₂ and N deposition, and K would likely become a limiting nutrient under increasing N deposition in subtropics.

Introduction

Mineral elements are important for plant growth and ecosystem function [1]. Base cations (potassium, K; calcium, Ca; magnesium, Mg) play a vital role in the capacity of buffering against acidity changes through exchange reactions [2]. They can also help plants against different stresses, such as drought, salinity and high temperature [3,4]. Trace metal cations (aluminium, Al; copper, Cu; manganese, Mn) are important both as micronutrients (10⁻⁵%~10⁻³%) [5] and as toxins when at high levels [6]. It is crucial to obtain sufficient concentrations of nutrient elements and maintain relatively stable stoichiometry in plant tissues for health [7,8]. Global change induced by human activities, such as increasing atmospheric carbon dioxide (CO₂)
concentration and nitrogen (N) deposition, has profoundly altered the biogeochemical cycles of several elements [9,10]. However, we know little the influence of increasing CO₂ and N deposition on these mineral elements in plants.

The increasing anthropogenic atmospheric CO₂ concentration stimulates plant growth, which increases C storage on land [11]. The extent to which elevated CO₂ increases plant growth, however, can be controlled or modified by available mineral elements in soil [12]. Elevated CO₂ often increases carbohydrates in plants and might logically be expected to lead to a decrease in the mineral element concentrations in plant tissues [10]. The nutrient dilution may preclude the positive effects of elevated CO₂ on plant growth [13,14]. Consequently, the interaction between CO₂ and nutrient status in plants has significant implications to the responses of forests to global change. While a frequent observation is that plants grown under elevated CO₂ typically have reduced tissue concentrations of N [15], the changes of other mineral element concentrations under elevated CO₂ were much more complex [16]. Using a meta-analysis method, Duval et al. [17] suggested that mineral elements were clearly different in response to elevated CO₂. Unfortunately, subtropical and tropical forests were not well represented in this meta-analysis. Our previous study has revealed that N and P concentrations in plants were not decreased by elevated CO₂ in subtropics, and instead P concentrations in plants positively responded to elevated CO₂ [18]. The results that challenged the assumption of declines in plant nutrient concentrations under elevated CO₂ [10], raise the question: what would happen to other mineral elements in response to elevated CO₂ in subtropical forests? As subtropical and tropical forests are characterized with multiple-nutrient limitation [19,20], the understanding of plant mineral elements in response to elevated CO₂ is critical for a better modeling plant productivity and biogeochemical cycling in forest ecosystems.

The increasing atmospheric deposition of N-containing compounds could have a pronounced effect on plants in response to elevated CO₂ [21,22]. Enhanced N deposition is associated with the accelerated loss of soil base cations, mobilization of heavy metal elements or lowered concentrations of base cations in forest ecosystems [23,24], and hence leads to nutrient imbalance in plant tissues. Through a meta-analysis, Lucas et al. [2] also suggested that foliar base cations consistently decreased following N addition over periods less than five years. These studies, however, separately treated the effects on nutrient status for N addition and CO₂ increase, and did not combine the effects of N addition with elevated CO₂ except for a few ones [25,26]. As air pollution and climate change are closely linked [25], the information on how N addition affects the dynamics of mineral elements in plant tissues under elevated CO₂ could lead to the development of a better perspective on plant nutrients in the contemporary complex environment.

Many studies on plant mineral elements in response to elevated CO₂ and N deposition focused on leaves [2]. However, different plant organs have different responses to elevated CO₂ and N deposition. For example, as leaves are highly metabolically active, the strength of regulatory control over elements would be stronger in leaves than in roots [27,28]. Therefore, we examined the responses of mineral elements in different plant organs (leaves and roots) to elevated CO₂ and N addition. Previous studies also have reported that the responses of terrestrial plants to elevated CO₂ and N deposition were species-specific, potentially driving a shift of the inter-specific competitive interactions and inducing species composition changes [29,30]. Therefore, it is necessary to examine the responses of multiple species to elevated CO₂ and N addition. However, there are big challenges in conducting the research of elevated CO₂ in mature forests due to their large stature and biological complexity [31]. The adult tree may be less responsive to environmental changes than the seedling. Thus, we used open-top chambers to study the effects of elevated CO₂ and N addition on the mineral elements (K, Ca, Mg, Al, Cu and Mn) in leaves and roots of the seedlings in five subtropical tree species over five years.
The five tree species are native to the study area and widely spread, including *Acmena acuminatissima* (Blume) Merr. et Perry (*A. acuminatissima*), *Syzygium hancei* Merr. et Perry (*S. hancei*), *Castanopsis hystrix* Hook.f. & Thomson ex A.DC (*C. hystrix*), *Obornsia pinnata* (Lour.) Merr. (*O. pinnata*) and *Schima superba* Gardn. Champ. (*S. superba*). The objectives of this study were to examine how elevated CO2 and N addition would influence plant mineral elements among the five tree species.

**Materials and Methods**

**Ethics statement**

The study site was owned by South China Botanical Garden, Chinese Academy of Sciences (CAS). The study was approved by South China Botanical Garden, CAS. All necessary permits were obtained for the described studies. The study did not involve endangered or protected species.

**Study site**

The study was carried out at South China Botanical Garden, CAS, Guangzhou City, Guangdong Province, China (23°20′N and 113°30′E). The area is characterized by a monsoon and humid climate. The mean annual temperature is 21.5°C, and the mean relative air humidity is 77%. The annual precipitation ranges from 1600 mm to 1900 mm with a distinct seasonal pattern, of which about 80% falls from April to September (wet season) and 20% occurs from October to March (dry season). The N deposition was high at our experimental site, with about 56 kg ha⁻¹ yr⁻¹ for the wet N deposition measured in 2006 [32].

**Open-top chamber design**

Ten open-top chambers were set up in an open space being exposed to full light and rain. Each chamber had a 3-m diameter, a 0.7-m deep below-ground part and a 3-m high above-ground part (adjusted to 4.5 m later). The below-ground part was delimited by brick walls in order to prevent any lateral or vertical water and/or element fluxes with the outside surrounding soils. Three holes at the bottom of the walls were connected to stainless steel water collection boxes. The above-ground part was wrapped with impermeable and transparent plastic sheets, leaving the top completely open. In the treatments with elevated CO2, an additional CO2 came from a tank, and was distributed by a transparent pipe that entwined the inner wall of the chamber in a snake shape at the height of 0.5–2.5 m. The pipe had pinholes at 1 cm intervals. The pipe was connected to a fan to ensure that CO2 was equally distributed in the entire chamber. The additional CO2 was applied daily from 8:00 am to 5:00 pm except for rainy days. The flux of CO2 from the tank was controlled by a flow meter to reach a target concentration of CO2 inside the chambers. The CO2 concentrations on the five planes (0.5, 1.0, 1.5, 2 and 2.5 m in height) in the chambers were monitored once a month using a Licor-6400 (LI-COR Inc., Lincoln, NE, USA).

**Experiment design**

Soils were collected from a nearby evergreen broad-leaved forest after harvesting in March 2005. Three different soil layers (0–20 cm, 20–40 cm and 40–70 cm) were placed into the belowground part of the chambers correspondingly after being homogenized separately. The bedrock was sandstone and shale. Soils were classified as ultisols following the United States Department of Agriculture (USDA) soil classification system [33].
Six native and widely spread tree species in southern China were chosen. They were *Acmena acuminatissima* (Blume) Merr. et Perry (*A. acuminatissima*), *Syzygium hancei* Merr. et Perry (*S. hancei*), *Castanopsis hystrix* Hook.f. & Thomson ex A.DC (*C. hystrix*), *Ormosia pinnata* (Lour.) Merr. (*O. pinnata*), *Schima superba* Gardn. Champ. (*S. superba*) and *Pinus massoniana* Lamb. (*P. massoniana*). Eight one- to two-year old seedlings for each tree species were randomly planted with inter-specific mixtures in each chamber at the density of 0.15 m² plant⁻¹. As *P. massoniana* died in the second year of our experiment, we studied the other five tree species in this experiment.

From April 2005, four treatments with two levels of CO₂ concentrations (elevated CO₂ and ambient CO₂) and two levels of N additions (with and without N fertilizer) were randomly applied to the ten chambers. Due to the logistically challenging to maintain the treatments with elevated CO₂, it is expected that there would be more variations in the treatments with elevated CO₂ than in those with ambient CO₂. In the face of limited resources, the treatments with elevated CO₂ replicated three times, while those with ambient CO₂ had two replications. That is, three chambers received an elevated CO₂ with N fertilizer (CN), three chambers did an elevated CO₂ without N fertilizer (CC), two chambers did an ambient CO₂ with N fertilizer (NN), and finally two chambers served as controls (ambient CO₂ without N fertilizer (CK). The elevated CO₂ treatments had a concentration of CO₂ at about 700 μmol CO₂ mol⁻¹. The N fertilized treatments were conducted by spraying once a week with a total amount of NH₄NO₃-N at 100 kg N ha⁻¹ yr⁻¹.

**Sample collection and measurement**

The initial soil chemical properties were measured before the experiment (See Table 1). Plant samples were collected from *A. acuminatissima*, *S. hancei*, *C. hystrix*, *O. pinnata*, and *S. superba*. One seedling for each species was randomly harvested by carefully digging out of the ground at the end of December in each year during 2005 to 2009. The majority of root biomass was collected. The removed soil was refilled into the holes left from the harvested trees. We collected the mature leaf and root samples from the harvested trees in December from 2005 to 2009 for the analysis of the mineral elements (K, Ca, Mg, Al, Cu and Mn). Plant samples were finely ground (0.25 mm) after being dried at 70°C for 72 h. The concentrations of K, Ca, Mg, Al, Cu and Mn were measured by inductively coupled plasma atomic emission spectroscopy (ICP, Optima-2000 DV, PerkinElmer, USA) after HNO₃ digestion.

**Statistical analysis**

Normality of the variables was examined with the Kolmogorov-Smirnov test, and the homogeneity of variance was tested with the Levene’s test. Data were logarithmically transformed when normality and homogeneity of variances were not confirmed. We analyzed data by repeated measures ANOVA using the following mixed linear model for each plant organ: Dependent variables = S + C + N + S×C +S×N + C×N + S×C×N, where S was the effect of different species, C was the effect of the CO₂ treatments, and N was the effect of the N treatments. The effect of the chambers was a random factor in the model. Although there were just two replications for CK and NN, more statistical power could be gained when data were analyzed with repeated measures ANOVA. When there was a significant interaction of the CO₂ treatments and N treatments, the differences between the four treatments (CK, NN, CC and CN) were further analyzed using Tukey multiple comparison test (HSD). The differences were considered to be statistically significant at $P < 0.05$. Data analyses were performed by the SAS software (SAS Institute Inc., Cary, NC, USA).
Results

Base cations in tree species

Across all the five tree species, the concentrations of K, Ca and Mg were relatively higher in the leaves (7.0 mg g⁻¹ for K, 7.4 mg g⁻¹ for Ca and 1.3 mg g⁻¹ for Mg) than in the roots (2.5 mg g⁻¹ for K, 6.3 mg g⁻¹ for Ca and 0.7 mg g⁻¹ for Mg) (Figs. 1 and 2 and S1 Data). The effects of elevated CO₂ on the base cations did not vary with plant organs (Table 2 and S1 Table). However, N addition significantly reduced K concentrations both in the leaves and roots (Table 2). The responses of Ca concentrations to N addition were different between roots and leaves, with some decreases in the roots but not in the leaves (Table 2).

The base cations significantly varied with species and the sampling time (Table 2). The effects of elevated CO₂ on the concentrations of the base cations largely depended on tree species (Table 2). Elevated CO₂ led some decreases in the base cations of *Acmena acuminatissima* (Blume) Merr. et Perry (*A. acuminatissima*), *Ormosia pinnata* (Lour.) Merr. (*O. pinnata*) and *Syzygium hancei* Merr. et Perry (*S. hancei*), while it did some increases in those of *Castanopsis hystrix* Hook.f. & Thomson ex A.DC (*C. hystrix*). Specifically, elevated CO₂ significantly decreased K concentrations in the leaves of *A. acuminatissima* in 2005 and the roots of *S. hancei* in 2009, Ca concentrations in the leaves and roots of *S. hancei* and *O. pinnata*, and Mg in the roots of *S. hancei* (Figs. 1 and 2). On the contrary, elevated CO₂ significantly increased the K concentrations in the roots of *C. hystrix* during the experimental period and those in its leaves in 2008 (Figs. 1 and 2). N addition consistently decreased K concentrations among the five tree species (Table 2). There were significant influences of N addition on Ca and Mg concentrations of *Schima superba* Gardn. Champ. (*S. superba*) and *O. pinnata*. To be specific, the Ca concentrations in the leaves of *S. superba* responded positively to the NN treatments in the early period of this experiment before 2007 (Fig. 1), while the lower Ca concentrations were found in the roots of *O. pinnata* under N addition after 2007 (Fig. 2). The Mg concentrations were significantly decreased by N addition in the roots of *S. superba* in 2007 and 2008.

Metal cations in tree species

The mean Al concentration across the five tree species was relatively greater in the roots (2.45 mg g⁻¹) than in the leaves (0.54 mg g⁻¹) (Figs. 3 and 4 and S1 Data). The averaged Cu concentration was 8.92 mg kg⁻¹ for leaves and 6.60 mg kg⁻¹ for roots. The mean Mn concentration was greater in the leaves (178 mg kg⁻¹) than in the roots (19 mg kg⁻¹). Relative to the leaves, Al concentrations in the roots across the five tree species tended to be lower under elevated CO₂ (*P = 0.079*) (Table 2). Elevated CO₂ significantly increased Cu concentrations in the roots by 18% across the five tree species (Table 2). The tree species had significantly influences on the metal cations (Table 2). The metal cations greatly varied with the sampling time (Table 2). The effects of elevated CO₂ on the metal
cations depended on tree species. Elevated CO₂ significantly decreased Al concentrations in the roots of *A. acuminatissima* at the beginning of this experiment (2005), and increased Mn concentrations in its roots in 2007 and 2008. For *C. hystrix*, elevated CO₂ tended to increase Cu concentrations in the roots after 2005, and Mn concentrations in the leaves and roots (Figs. 3 and 4). There were some increases in Cu concentrations in the roots of *O. pinnata* but a decrease in the Mn concentrations in its leaves under elevated CO₂. Elevated CO₂ increased Al concentrations in the leaves of *S. hancei*, and the effects were stronger with time (Fig. 3). *S. superba* exhibited higher foliar Mn concentrations in the CC treatments, but the positive effects of the CC treatment tended to be muted with time (Fig. 3). On the other hand, N addition had no influence on Cu concentrations in the five tree species, but it had significant effects on the Al and Mn concentrations in *A. acuminatissima*, *O. pinnata* and *S. hancei*. Specifically, N addition significantly lowered Al concentrations in the roots of *A. acuminatissima* in 2005 (Fig. 4).

Fig 1. Concentrations of base cations in the leaves of five subtropical tree species exposed to different CO₂ and N treatments from 2005 to 2009. Each error bar is one standard error. CK, control; NN, ambient CO₂ with N fertilizer; CC, elevated CO₂ without N fertilizer; CN, elevated CO₂ with N fertilizer. (a-c) *A. acuminatissima*; (d-i) *S. hancei*; (g-i) *C. hystrix*; (j-l) *O. pinnata*; (m-o) *S. superba.*

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**Discussion**

**Effects of elevated CO₂ on plant mineral elements**

The concentrations of plant elements were expected to decline if the uptake of elements was not improved at the same rate as dry matter accumulation under elevated CO₂ [10]. Our results showed some declines in the concentrations of the base cations and metal cations under elevated CO₂. However, the declines did not occur for the whole experimental time. We found no changes or even some increases in the mineral elements in plants under elevated CO₂. Our

*O. pinnata* had greater foliar Al concentrations under N addition but lower foliar Mn concentrations in 2009. For *S. hancei*, there were increases in foliar Al concentrations and in Mn concentrations in the roots under N addition.
results were consistent with other studies, which reported little or even some positive responses of mineral elements in plants to elevated CO2 [6,26,34]. No decline in plant mineral elements could be explained by the following factors. First, the greater soil moisture content from decreased evapotranspiration under elevated CO2 detected in our experiment [35] could stimulate soil microbial processes and then facilitate litter decomposition and mineral weathering [36]. Second, plant root growth was increased under elevated CO2 in our experiment [37], which could improve nutrient uptake. Moreover, elevated CO2 could indirectly increase the release of cations from the mineral weathering by enhancing carbonic acid [38], which was confirmed by the increased inorganic C leaching and higher cation concentrations in soil water under elevated CO2 in our experiment [39,40]. Therefore, these mechanisms could be responsible for no changes or some increases in the concentrations of the mineral elements even with biomass stimulation under elevated CO2 [37].

Across the five tree species, our results showed that leaves were less responsive to elevated CO2 than roots with regard to the metal cations. This provided the evidence to the suggestion that elements in leaves were relatively constrained to maintain metabolic activity when compared with roots [28]. The lower Al concentrations in the roots under elevated CO2 could be explained by the growth dilution due to the great allocation of C to root growth [37]. However, it did not appear that the growth dilution was the primary factor that influencing Al concentrations as other metal cations (Cu and Mn) did not decrease under elevated CO2. The decreased Al concentrations and increased Cu concentrations in the roots suggest that there would be a biological regulation of metal cations [6]. The down-regulation of Al concentrations in the roots suggests that elevated CO2 would help plants to alleviate Al toxicity in the contaminated systems.

Compared with the other tree species, Schima superba Gardn. Champ. (S. superba) displayed a competitive advantage at biological regulation of nutrient balance under elevated CO2 alone, given less changes in the concentrations of the mineral elements.
Our results showed that N addition led to decreases in the concentrations of base cations, especially K, and increases in Al and Mn in some tree species. Previous studies have reported the elements (e.g. Ca and Mg) in plants were lowered by N addition [24,41], which was partly consistent with our study. The shifts in the mineral elements of the seedlings could be explained by the changes in soil chemistry with increasing N inputs. High N deposition often resulted in a decline in base cations and an increase in soluble metal cations in soil solution [42]. The consequence of the decline in base cations was well reflected by the decrease in K concentrations in the five tree species in our study. Several studies have emphasized the importance of K as a co-limiting nutrient in forest ecosystems as the increased supply of other nutrients [20,43]. Our results also highlight the need to consider K limitation to plant growth under increasing N deposition. On the other hand, in the same experiment, the metal cations (Al and Mn)
The mobilization of Al and Mn may be responsible for the increased Al and Mn concentrations in *Syzygium hancei* Merr. et Perry (*S. hancei*) or *Ormosia pinnata* (Lour.) Merr. (*O. pinnata*).

When considering Ca concentrations, roots responded more strongly to N addition than leaves. The results would appear to further support the argument that leaves were less sensitive indicators of soil nutrient availability than roots [28]. On the contrary, K concentrations were decreased by N addition in both the leaves and roots. This probably suggested a restricted mobilization of K from roots towards leaves when K was shortage under N addition. As K dynamics appear to be unique among the base cations (Ca and Mg) [43], further research is necessary to emphasize K cycles under increased N deposition.

When compared with the other tree species, *Castanopsis hystrix* Hook.f. & Thomson ex A. DC (*C. hystrix*) was less responsive to high N availability during the experiment. This is
corresponding to no significant effects of N addition on the annual NPP of C. hystrix [44]. Further studies are needed to understand the underlying mechanisms of the adaptation of C. hystrix to increasing N deposition.

**Interactive effects between elevated CO2, N addition and the sampling time**

The mineral elements of plants in response to elevated CO2 and N addition varied with the sampling time, as indicated by their interactions (Table 2 and S1 Table). As mentioned above, elevated CO2 enhanced base cations in soils, and N addition resulted in the mobilization of metal cations. However, these effects had a lag time to support the faster growth under elevated CO2 at the beginning of this experiment, thus resulting in lower mineral elements in plants. Moreover, the effects of elevated CO2 on forests would not be sustained over time [45]. The increased leached amounts of base cations induced by elevated CO2 in the same experiment were found to be weakening with time [40]. Thus, elevated CO2 would lead to nutrient limitation to plant growth in the long-time. Finally, the variations between annual precipitations might also influence plant mineral elements in response to elevated CO2 and N addition. More studies on the relationships between precipitation and the mineral elements in plants are needed.

**Conclusions**

Elevated CO2 had more influences on the mineral elements in the roots than in the leaves. Elevated CO2 did not lead to a consistent decline in plant mineral elements in this experiment. The concentrations of K, Al, Cu and Mn were increased by elevated CO2 in some tree species. N addition led to a decrease in K across the five tree species. The response of plant mineral elements to elevated CO2 and N addition varied with tree species, with S. superba and C. hystrix less responsive to elevated CO2 and N addition alone, respectively. Our results have important implications on the biogeochemical cycles and species composition in subtropical forests under elevated CO2 and N addition. In the future, the availability of K in the lateritic soils would probably constrain plant growth in response to increasing N deposition in our region.

**Supporting Information**

**S1 Data.** Concentrations of mineral elements of five subtropical tree species exposed to different CO2 and N treatments from 2005 to 2009. CK, control; NN, ambient CO2 with N fertilizer; CC, elevated CO2 without N fertilizer; CN, elevated CO2 with N fertilizer. (CSV)

**S1 Table.** Statistical results from repeated measures ANOVA on the effects of different species (S), carbon dioxide (C) and nitrogen (N) treatments and their interactions on the concentrations of mineral elements of five subtropical tree species. (DOCX)

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**Author Contributions**

Conceived and designed the experiments: JL GZ DZ WH. Performed the experiments: SL GC XF WH. Analyzed the data: WH JL. Contributed reagents/materials/analysis tools: JL GZ DZ. Wrote the paper: WH JL.
References
1. Marschner H (1995) Mineral nutrition of higher plants. New York: Academic Press. NY.
2. Lucas RW, Klaminder J, Futter MN, Bishop KH, Egnell G, Laudon H, et al. (2011) A meta-analysis of the effects of nitrogen additions on base cations: Implications for plants, soils, and streams. For Ecol Manage 262: 95–104.
3. Likens GE, Driscoll CT, Buso DC, Siccama TG, Johnson CE, Lovett GM, et al. (1998) The biogeochemistry of calcium at Hubbard Brook. Biogeochemistry 41: 89–173.
4. Cakmak L (2005) The role of potassium in alleviating detrimental effects of abiotic stresses in plants. J Plant Nutr Soil Sci 168: 521–530.
5. Zhang JS (2006). Plant Physiology. Beijing: Higher Education Press.
6. Natali SM, Sanudo-Wilhelmy SA, Lerdau MT (2009) Plant and soil mediation of elevated CO2 impacts on trace metals. Ecosystems 12: 715–727.
7. Ågren GI (2008) Stoichiometry and nutrition of plant growth in natural communities. Annu Rev Ecol Evol Syst 39: 153–170.
8. Han WX, Fang JY, Reich PB, Woodward FI, Wang ZH (2011) Biogeography and variability of eleven mineral elements in plant leaves across gradients of climate, soil and plant functional type in China. Ecol Lett 14: 788–796. doi: 10.1111/j.1461-0248.2011.01641.x PMID: 21692962
9. Vitousek PM, Aber JD, Howarth RW, Lienk GE, Matson PA, Schindler DW, et al. (1997) Human alteration of the global nitrogen cycle: sources and consequences. Ecol Appl 7: 737–750.
10. Loladze I (2002) Rising atmospheric CO2 and human nutrition: toward globally imbalanced plant stoichiometry? Trends Ecol Evol 17: 457–461.
11. Norby RJ, DeLucia EH, Gielen B, Califapetra C, Giardina CP, King JS, et al. (2005) Forest response to elevated CO2 is conserved across a broad range of productivity. Proc Natl Acad Sci USA 102:18052–18056. PMID: 16612381
12. Hungate BA, Dukes JS, Shaw MR, Luo YQ, Field CB (2003) Nitrogen and climate changes. Science 302: 1512–1513. PMID: 1465831
13. Woodin S, Graham B, Killick A, Skiba U, Cresser M (1992) Nutrient limitation of the long term response of heather Calluna vulgaris (L.) hull to CO2 enrichment. New Phytol 122: 635–642.
14. Reich PB, Hobbie SE, Lee T, Ellsworth DS, West JB, Tilman D, et al. (2006) Nitrogen limitation constrains sustainability of ecosystem response to CO2. Nature 440: 922–925. PMID: 16612381
15. Taub DR, Wang XZ (2008) Why are nitrogen concentrations in plant tissues lower under elevated CO2? A critical examination of the hypothesis. J Integr Plant Biol 50: 1365–1374. doi: 10.1111/j.1744-7909.2008.00754.x PMID: 19017124
16. Johnson DW, Hungate BA, Dijkstra P, Hymus G, Hinkley CR, Stiling P, et al. (2003) The effects of elevated CO2 on nutrient distribution in a fire-adapted scrub oak forest. Ecol Appl 13: 1388–1399.
17. Duval BD, Blankinship JC, Dijkstra P, Hungate BA (2012) CO2 effects on plant nutrient concentration depend on plant functional group and available nitrogen: a meta-analysis. Plant Ecol 213: 505–521.
18. Liu JX, Huang WJ, Zhou GY, Zhang DQ, Liu SZ, Li YY (2013) Nitrogen to phosphorus ratios of tree species in response to elevated carbon dioxide and nitrogen addition in subtropical forests. Glob Chang Biol 19: 208–216. doi: 10.1111/gcb.12022 PMID: 23504732
19. Vitousek PM, Sanford RL (1986) Nutrient cycling in moist tropical forest. Annu Rev Ecol Syst 17: 137–167.
20. Santiago LS, Wright SJ, Harms KE, Yavitt JB, Korine C, Garcia MN, et al. (2012) Tropical tree seedling growth responses to nitrogen, phosphorus and potassium addition. J Ecol 100: 309–316.
21. Nadelhoffer KJ, Emmett BA, Gundersen P, Kjennaas OJ, Koopmans CJ, Schleppi P, et al. (1999) Nitrogen deposition makes a minor contribution to carbon sequestration in temperate forests. Nature 398: 145–148.
22. Hofmockel KS, Gallet-Budynek A, McCarthy HR, Currie WS, Jackson RB, Finzi A (2011) Sources of increased N uptake in forest trees growing under elevated CO2: results of a large-scale 15N study. Glob Chang Biol 17: 3338–3350.
23. Watmough SA, Dillon PJ (2003) Base cation and nitrogen budgets for a mixed hardwood catchment in south-Central Ontario. Ecosystems 6: 675–693.
24. Elvir JA, Wiersma GB, Day ME, Greenwood MS, Fernandez IJ (2006) Effects of enhanced nitrogen deposition on foliar chemistry and physiological processes of forest tree at the Bear Brook Watershed in Maine. For Ecol Manage 221: 207–214.
25. Bytnerowicz A, Omura K, Paolletti E (2007) Integrated effects of air pollution and climate change on forests: A northern hemisphere perspective. Environ Pollut 147: 438–445. PMID: 17034915
26. Marinari S, Calfapietra C, De Angelis P, Mugnozza GS, Grego S (2007) Impact of elevated CO2 and nitrogen fertilization on foliar elemental composition in a short rotation poplar plantation. Environ Pollut 147: 507–515. PMID: 17084005

27. Garrish V, Cernusak LA, Winter K, Turner BL (2010) Nitrogen to phosphorus ratio of plant biomass versus soil solution in a tropical pioneer tree, Ficus insipida. J Exp Bot 61: 3735–3748. doi: 10.1093/jxb/erq183 PMID: 20591897

28. Schreeg LA, Santiago LS, Wright SJ, Turner BL (2014) Stem, root, and older leaf N:P ratios are more responsive indicators of soil nutrient availability than new foliage. Ecology 95: 2062–2068. PMID: 25230458

29. Xia JY, Wan SQ (2008) Global response pattern of terrestrial plant species to nitrogen addition. New Phytol 179: 428–439. doi: 10.1111/j.1469-8137.2008.02488.x PMID: 19086179

30. Reich PB, Tilman D, Craine J, Ellsworth D, Tjoelker MG, Knops J, et al. (2001) Do species and functional groups differ in acquisition and use of C, N and water under varying atmospheric CO2 and N availability regimes? A field test with 16 grassland species. New Phytol 150: 435–448.

31. Cernusak LA, Winter K, Dalling JW, Holtum JAM, Jaramillo C, Körner C, et al. (2013) Tropical forest responses to increasing atmospheric CO2 current knowledge and opportunities for future research. Funct Plant Biol 40: 531–551.

32. Liu JX, Zhang DQ, Zhou GY, Fairevre-Vuillin B, Deng Q, Wang CL (2008) CO2 enrichment increases nutrient leaching from model forest ecosystems in subtropical China. Biogeosciences 5: 1783–1795.

33. Buol SW, Southard RJ, Graham RC, McDanieel PA (2003) Soil genesis and classification, fifth edition. Ames: Iowa State Press. Iowa.

34. Johnson DW, Cheng W, Joslin JD, Norby RJ, Edwards NT, Todd DE JR (2004) Effects of elevated CO2 on nutrient cycling in a sweetgum plantation. Biogeochemistry 69: 379–403.

35. Deng Q, Zhou G, Liu J, Liu S, Duan H, Zhang D (2010) Responses of soil respiration to elevated carbon dioxide and nitrogen addition in young subtropical forest ecosystems in China. Biogeosciences 7: 315–328.

36. Niklaus PA, Spinnler D, Körner C (1998) Soil moisture dynamics of calcareous grassland under elevated CO2. Oecologia 117: 201–208.

37. Zhao L, Zhou GY, Zhang DQ, Duan HL, Liu JX (2011) Effects of elevated CO2 concentration and nitrogen deposition on the biomass accumulation and allocation in south subtropical main native tree species and their mixed communities (in Chinese with English abstract). Chin J Appl Ecol 22: 1949–1954.

38. Andrews JA, Schlesinger WH (2001) Soil CO2 dynamics, acidification, and chemical weathering in a temperate forest with experimental CO2 enrichment. Glob Biogeochem Cycle 15: 149–162.

39. Liu JX, Xu ZH, Zhang DQ, Zhou GY, Deng Q, Duan HL, et al. (2011) Effects of carbon dioxide enrichment and nitrogen addition on inorganic carbon leaching in subtropical model forest ecosystems. Ecosystems 14: 683–697.

40. Liu JX, Zhang DQ, Huang WJ, Zhou GY, Li YL, Liu SZ (2014) Quantify the loss of major ions induced by CO2 enrichment and nitrogen addition in subtropical model forest ecosystems. J Geophys Res 119: 676–686.

41. Bowman WD, Cleveland CC, Halada L, Hresko J, Baron JS (2008) Negative impact of nitrogen deposition on soil buffering capacity. Nat Geosci 1: 767–770.

42. Turner BL, Yavitt JB, Harms KE, Garcia MN, Romero TE, Wright SJ (2013) Seasonal changes and treatment effects on soil inorganic nutrients following a decade of fertilizer addition in a lowland tropical forest. Soil Sci Soc Am J 77: 1357–1369.

43. Tripler CE, Kaushal SS, Likens GE, Walter MT (2006) Patterns in potassium dynamics in forest ecosystems. Ecol Lett 9: 451–466. PMID: 16623731

44. Yan JH, Zhang DQ, Liu JX, Zhou GY (2014) Interactions between CO2 enhancement and N addition on net primary productivity and water use efficiency in a mesocosm with multiple subtropical tree species. Glob Chang Biol 20: 2230–2239. doi: 10.1111/gcb.12501 PMID: 24339232

45. Xu ZH, Chen CR, He JZ, Liu JX (2009) Trends and challenges in soil research 2009: linking global climate change to local long-term forest productivity. J Soils Sediments 9: 83–88.