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RESEARCH ARTICLE

Does nitrogen fertilization alter the scaling relationships of multinutrients in tree organs? Evidence from Chinese hickory (*Carya cathayensis*) saplings

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Abstract

Aims Nitrogen (N) enrichment from excessive fertilization influences the coupling of nutrients in terrestrial ecosystems, but whether it could alter the scaling relationships between multinutrients [including macronutrients: N, phosphorus (P), potassium, calcium (Ca), magnesium (Mg) and micronutrients: manganese (Mn), iron (Fe) and zinc] remains unclear.

Methods We conducted an control experiment using two-year-old Chinese hickory (*Carya cathayensis*) saplings in hydroponics system, with six levels of N fertilization, to evaluate variations in scaling relationships of multinutrient concentrations in vegetative organs of Chinese hickory under consistent N fertilization.

Results Correlational relationships between multinutrients concentrations were most significant in leaves, while stems and roots were less related. Micronutrients, in particular, were mostly negatively related to macronutrients. Significant allometric relationships were detected among multinutrients, but the response patterns were both organ- and nutrient-dependent. For example, positive allometric relationships were detected in N versus other macronutrients while negative allometric relationships were observed with N related to micronutrient response. Leaves generally presented more positive allometric relationships than negative relationships. Beyond expectation, increasing N fertilization minimally altered the scaling exponents between multinutrients, except stem N-Mg, root Mn-Fe, root P-Ca, and leaf P-Ca.

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Conclusions Chinese hickory saplings displayed relatively high nutrient homeostasis when excessive N fertilizer was applied. These results will enhance our understanding of organ-dependent allometric relationships between multinutrients in forest trees and may offer new insights into how plants develop adaptive functional traits to increasing N fertilization.

Keywords Nitrogen fertilization · Macronutrients · Micronutrients · Vegetative organs · Scaling relationships

Introduction

Plants need at least 17 elemental nutrients, all of which are equally important and irreplaceable in supporting plant growth and metabolic processes (Watanabe et al. 2007; Valentinuzzi et al. 2018; Tiziani et al. 2020a, b). To obtain optimal metabolic activity and maximize growth, plants must maintain the balance of these nutrients under different stressors (Schreeg et al. 2014; Yan et al. 2016). Given the tight functional relationship between nutrients, their concentrations in tissues often show a closely stoichiometric scaling relationship, which can be quantified by a power function as $Y = \alpha X^{\beta}$, where Y and X are interdependent variables, such as leaf nitrogen (N) and leaf phosphorus (P), α is the allometric constant, and β is the scaling exponent (also called allometric coefficient) (Hunt 1990). The scaling exponent β depicts the alternative accumulation of one nutrient compared to the other. When $\beta = 1$, the scaling relationship between X and Y is isometric, meaning the two nutrients have the same accumulation rate; when $\beta > 1$, the scaling relationship is allometrically positive, meaning that nutrient Y accumulates more than nutrient X; and when $\beta < 1$, the scaling relationship is allometrically negative, whereby nutrient Y accumulates less than nutrient X (Shingleton 2010). Thus, scaling relationships indicate the proportionality of pairwise traits and are important to our fundamental ecological understanding of plant response to external stressors.

Prior studies have documented scaling relationship of plant N to P for their frequently constraint on plant growth and primary productivity across ecosystems (Elser et al. 2007). Macronutrient cations [(potassium (K), calcium (Ca), and magnesium (Mg)], and micronutrient cations [here refers to manganese (Mn), iron (Fe) and zinc (Zn)] have received less attention, although they are essential in maintaining plant performance (Vitousek et al. 2010; Ågren and Weih 2012). For instance, K is closed related to the control of water conductance and transpiration (Sardans and Peñuelas 2015) while Mg is essential for leaf photosynthesis (Laing et al. 2000). The scaling relationships between macronutrients (N, P, K, Ca, Mg) and micronutrients (Mn, Fe, Zn) are equally important since the balances between them are essential to the optimal growth, development, and productivity of plants. The absence or imbalance of any macro- or micronutrient would result in nutrient deficiency (Sardans et al. 2008; Tiziani et al. 2021). But limited reports are available on the scaling relationships between macro- and micronutrients or multinutrients. Studies have postulated that scaling relationships of plant N-P appear to be insensitive to environmental conditions such as latitude, precipitation, and temperature, but these studies have been contrasted by others (Cheng et al. 2007; Yan et al. 2018; Tian et al. 2018). These contentious results regarding variation in plant N-P scaling exponents proposed an important question: How would scaling relationship between macro- and micronutrients respond to changes in environmental conditions? Especially since the homeostasis of these nutrients is important in modelling stoichiometric growth and nutrient cycles in terrestrial ecosystems (Peñuelas et al. 2013).

Nitrogen fertilizer consumption has consistently increased in the past two decades due to the growing utilization of N fertilization in agriculture, forestry, and other sectors (Stevens et al. 2018). Considering the huge atmospheric N deposition in subtropical regions (> 30 kg N ha^{-1} yr^{-1} each year), the total N input has greatly exceeded the N requirement of forested ecosystems in this area (Zhang et al. 2017; Song et al. 2017). Many studies have documented the profound negative affect of excessive N input on terrestrial ecosystems, including soil acidification and nutrient imbalance resulting from microbe-mediated N transformation (mineralization, nitrification, and denitrification) or exchanges of cations (Liu et al. 2011a, b; Zhu et al. 2015; Cusack et al. 2016; Bourgeois et al. 2019). At species level, superlative N input may greatly impact plants' ability to maintain homeostasis of multiple nutrients, which is a fundamental driver in tree growth and can be assessed by the variation of scaling exponents between any two nutrients (Sardans and Peñuelas 2015; Sardans et al. 2017). Empirical studies have documented diverse allometric scaling relationships for tree species under various conditions (Swetnam et al. 2016; Chen 2018). For instance, Duncanson et al. (2015) suggested that younger forests may take on a variety of allometries, but mature forests (tall forests) exhibit relatively consistent allometric patterns. Variations in scaling relationships could be a useful tool in predicting plant nutrient status and consequently the development of adaptive functional traits among organs (Allen et al. 2010). Therefore, it is critical to understand the allometric response of plant multinutrients when considering the expanding impact of N fertilization on forest ecosystems (Kou et al. 2018).

Homeostasis of elemental nutrients in different organs is a fundamental aspect of plant response and adaptation to the changing environmental conditions, but how it responds to N availability is not well addressed (Peng et al. 2016). Basically, leaves respond more actively to N additions than other plant organs, but data are still insufficient to understand if other vegetative organs like roots and stems can also maintain nutrient balance under excessive N input (Wang et al. 2016). In many cases, nutrient homeostasis in leaves has been evaluated by the variation of scaling relationships, which is helpful for revealing adaptation strategies of tree species regarding nutrient allocation and utilization (Schreeg et al. 2014). In stems and roots, the scaling relationship between multiple nutrients could also be altered by excessive N input, but the degree to which N-induced changes in scaling exponents in different plant organs remains understudied. Thus, manipulative experiments are warranted to test if organ-dependent differences exist in multinutrient scaling relationships under N fertilization.

Chinese hickory (*Carya cathayensis* Sarg., hereafter, hickory) is widely cultivated in Zhejiang Province, China, with a huge reputation for its highly nutritious nuts (Xu et al. 2016). But, recently, private landowners are using copious amounts of N fertilizers to increase yield, leading to not only unstable productivity, but also declines in nut quality, greatly impacting the economic development of hickory in China (Liu et al. 2011a, b). Therefore, it is of great economic importance to understand nutrient homeostasis of hickory and its allometric response to excessive N fertilization. In this context, we established a hydroponics experiment with two-year-old hickory saplings to test the effects of excessive N fertilization on scaling relationships between macro- and micronutrients in different plant organs. We tested two hypotheses: (i) The scaling relationship between macro- and micronutrients would differ between vegetative organs, leaves will have more allometric relationships than roots or stems because leaves are more active in metabolic processes. (ii) In response to excessive N fertilization, scaling relationships will be significantly altered in plant vegetative organs between macro- and micronutrients.

Materials and methods

Plant material and fertilizer solution

Chinese hickory saplings that were cultivated in the nursery of Tianze Hickory Company (Lin'an, Hangzhou) for two years were used to perform the N fertilization experiment. The saplings had an average height of 80.0 cm and a basal diameter of 6.5 mm and were observed to be in similar healthy condition. A total of 150 identical hickory saplings were chosen to conduct the N fertilization experiment. The selected saplings were transplanted into uniform plastic pots (height: 30.5 cm, top diameter: 25 cm) and filled with full-strength fertilizer solution. The fertilizer solution was composed of 1.25 mmol/L $Ca(NO_3)_2$, 0.5 mmol/L $Ca(H_2PO_4)_2$, 1.0 mmol/L K_2SO_4, 0.1 mmol/L $MgSO_4$, 12.5 µmol/L H_3BO_3, 1.0 µmol/L $MnSO_4$, 1.0 µmol/L $ZnSO_4$, 0.25 µmol/L $CuSO_4$, 0.1 µmol/L $(NH_4)_6Mo_7O_{24}$, and 10 µmol/L EDTA-Fe, with an initial pH of 5.7.

Experimental design

The experiment was performed within the artificial greenhouse at Zhejiang A& F University (Lin'an, Hangzhou). During the daytime, the greenhouse had natural sunlight with temperatures set at 30°C. At night, the temperature was set at 25°C without artificial light. The relative humidity was maintained at approximately 60% throughout the experiment (±5%). To begin the experiment, we first washed the roots of the hickory saplings with pure water to remove the attached soil and then transplanted them into the plastic pots filled them with a 12.5 L nutrient solution. Before initiating the N fertilization treatments, the hickories acclimated to the transplanting solution and greenhouse condition for one month to allow for adaptation to the experimental environment. The N fertilization experiment included 6 N treatments with 25 replications per treatment and conducted as a randomized complete block factorial design. The treatments were: a control with no N fertilization (N0), N1, N2, N3, N4 and N5 treatments represented by the addition of 0.5, 1.5, 2.5, 4 and 6 mmol N (calculated by $Ca(NO_3)_2$ concentration), respectively. The reason for using $Ca(NO_3)_2$ as the source for experimentally increasing N fertilization level is that $Ca(NO_3)_2$ has high solubility and limited effect on solution pH (Xie et al. 2022). During the experiment, the solution as well as N fertilizer were both regenerated every 7 days to ensure the effectiveness of nutrient solution, the pH was regulated with portable pH meter. Since the pH could be altered following the N additions, $Ca(OH)_2$ and HCl were used to regulate the pH of the nutrient solution to 5.7. Additionally, aeration of the nutrient solution in the plastic pots was maintained throughout the experiment with small air pump.

Sampling, measurement and chemical analysis

Upon removal of the saplings from the plastic pots, we washed them with deionized water to clean off any adhering solution and dried them with absorbent paper. We carefully separated the saplings into roots, stems, and leaves. All the samples were labeled and immediately chilled in iced coolers. Samples were oven-dried (105 °C for 1 h and 65 °C for 48 h) to a constant weight and finely ground with mixer mill (Retsch GmbH MM400, Haan, Germany). Total N concentration in plant vegetative organs (roots, stems, and leaves) was determined with a C-N auto analyzer (Elementar VarioMAX, Germany) while concentrations of total P, K, Ca, Mg, Mn, Fe and Zn were obtained by inductively coupled plasma-optical emission spectrometry (ICP-OES, PEO 5300 DV, Waltham, MA, USA) after digestion of the tissue samples in concentrated HNO_3.

Statistical analysis

Data normality (Kolmogorov–Smirnov-Test) were evaluated before conducting the subsequent statistical analyses. Partial correlation analysis was used to evaluate the relationship between macro- and micronutrients in different vegetative organs. Statistical analyses above were performed using SPSS 20.0 (IBM SPSS Statistics, U.S.A.) with the significance level set at $\alpha = 0.05$. Data were log-transformed to improve normality and reduce heteroscedasticity before conducting allometric analysis. Standardized major axis (SMA) regressions were used to test the allometric relationship between macro- and micro elements in different organs with the “smatr” package in R 3.3 (Warton et al. 2012). 95% confidence intervals (CI) of scaling exponents were also estimated to statistically determine if the relationship between two nutrients was isometric or allometric.

Results

Organ-dependent correlations between multiple nutrients

Regardless of organ, the nutrients were generally significantly correlated (19 out of 28 pairs), despite observed divergent patterns (Fig. 1A). Across organs, there was variation in the number of correlations, leaves were the most correlated (19 out of 28 pairs), followed by stems (14 out of 28 pairs), and roots were the least correlated (9 out of 28 pairs). Micronutrients were generally negatively correlated with macronutrients. Mn was especially negatively correlated with all the macronutrients in stems and leaves, except for stem Mg. Meanwhile, Zn was generally negatively correlated with macronutrients in roots, but was positively correlated with macronutrients in leaves (Figs. 1B, 2C, D).

Allometric relationships among macronutrients and their response to N fertilization

In roots, significant positive allometric relationships were found between N and K, and between P and K. While stem N and P nearly exhibited a significant isometric relationship. Significant negative allometric relationships were found between both P and Ca, and between K and Ca (Fig. 2 and Table S1). Increasing N fertilization significantly elevated only the scaling exponents of N to Mg in stems, while other scaling exponents were not impacted (Fig. 3). In stems, significant positive allometric relationships were found between N and P, K, and Ca, respectively. P also showed significant positive allometric relationships to K, but displayed a negative relationship to Ca. K showed a negative allometric relationship to Ca (Fig. 2 and Table S1). With increasing N additions, the scaling exponent of P to Ca decreased significantly, while

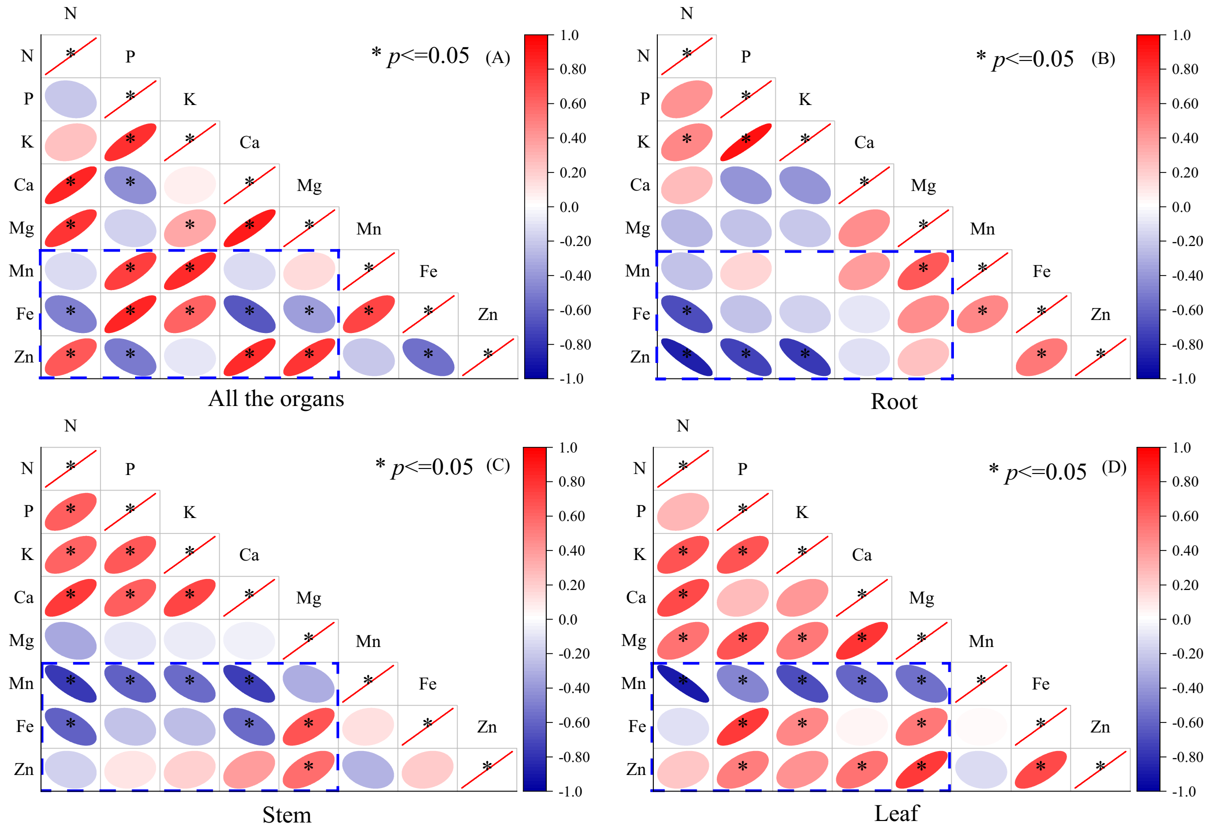

Fig. 1 Correlations between concentrations of multinutrients in combined and separate organs. The blue dotted boxes emphasize the correlational relationship between macro- and micronutrients ($n=6$)

other scaling exponents were not altered (Fig. 3). In leaves, strong and significant positive allometric relationships were found between N and P, K, Ca and Mg, respectively. P showed significant positive allometric response to both K and Ca, but a negative allometric relationship to Mg. Negative allometric relationship were also found between K and Ca, K and Mg, and Ca and Mg (Fig. 2 and Table S1). With increasing N additions, the scaling exponent of P to Ca was significantly reduced, but the scaling exponents between other macronutrients was not altered (Fig. 3).

Allometric relationships among micronutrients and their response to N fertilization

In roots, Mn and Fe exhibited a positive allometric relationship while Fe and Zn had a negative allometric relationship. In stems, no significant allometric relationships were found between micronutrients. In leaves, significant positive allometric relationships were found between Fe and Zn. Increasing N fertilization had no significant effects on the scaling exponents between micronutrients across organs except for the scaling exponent of N to Mg in roots, which was greatly enhanced by N fertilization (Fig. 4).

Allometric relationships between macro- and micronutrients and their response to N fertilization

Significant negative allometric relationships were found between macro- and micronutrients across organs. Surprisingly, not a single positive allometric relationship was detected. A total of 4 pairs of negative scaling relationships were found in roots, (Mg to Mn, N, and P, and K to Zn). In stems, 7 pairs of negative scaling relationships were detected (Mn to N, P, K, and Ca; Fe to Ca and Mg;

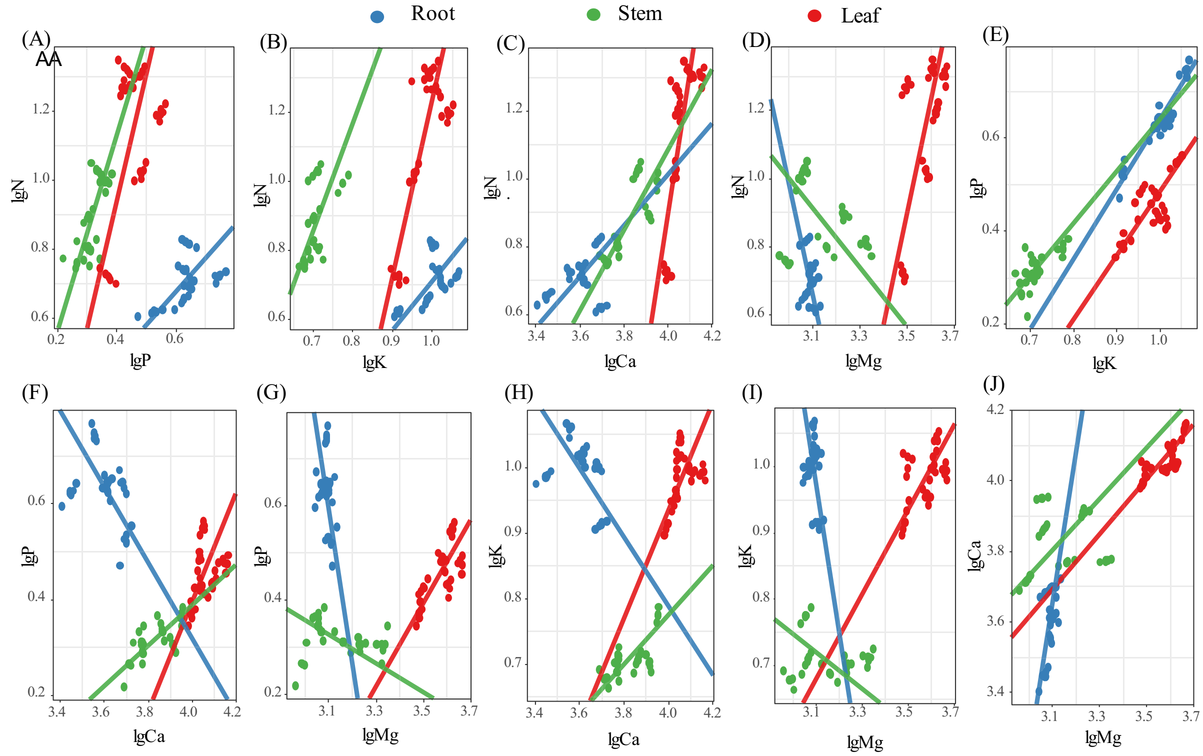

Fig. 2 Allometric relationships among macronutrients in different vegetative organs of hickory saplings using a standardized major axis (SMA) method ($n=6$). Root, stem and leaf are represented with blue, green and red circles, respectively. Allometric scaling exponent equals to slope of the regression line. Note that graph axes are log10 scaled

and Mg to Zn). In leaves, 10 pairs of negative scaling relationships were found and only 5 pairs were non-significant (N-Fe, K- Fe, Ca-Fe, N-Zn, and K-Zn). With increasing N fertilization, the scaling exponents between macro- and micronutrients were not significantly altered, although an increasing or decreasing trend can be observed (Figs. 5 and 6, Table S2).

Discussion

Identifying the allometric relationships among nutrients are critical for revealing the interactive mechanisms between plant traits and the utilization of related resources. It is also important for estimating alterations to forest dynamics from environmental changes (Li et al. 2008; Gargaglione et al. 2010; Pretzsch et al. 2015). Here, we evaluated the organ-dependent response of multinutrient allometric relationships to N fertilization using hickory saplings through a hydroponics system. We found extensive correlational relationships between multinutrients in leaves and stems, while less interdependence in roots. This indicates that macro- and micronutrients do affect each other and may migrate between different organs in hickory saplings. Noteworthy, root cation contents could be overestimated in hydroponics if exchange solutions were not used to desorb cations adsorbed on root apoplast, thus may affect the correlational relationships between multinutrients in roots. Besides, we found divergent allometric relationships among macro- and micronutrients, indicating that allometric relationships between nutrients were prevalent in hickory saplings at the organ level.

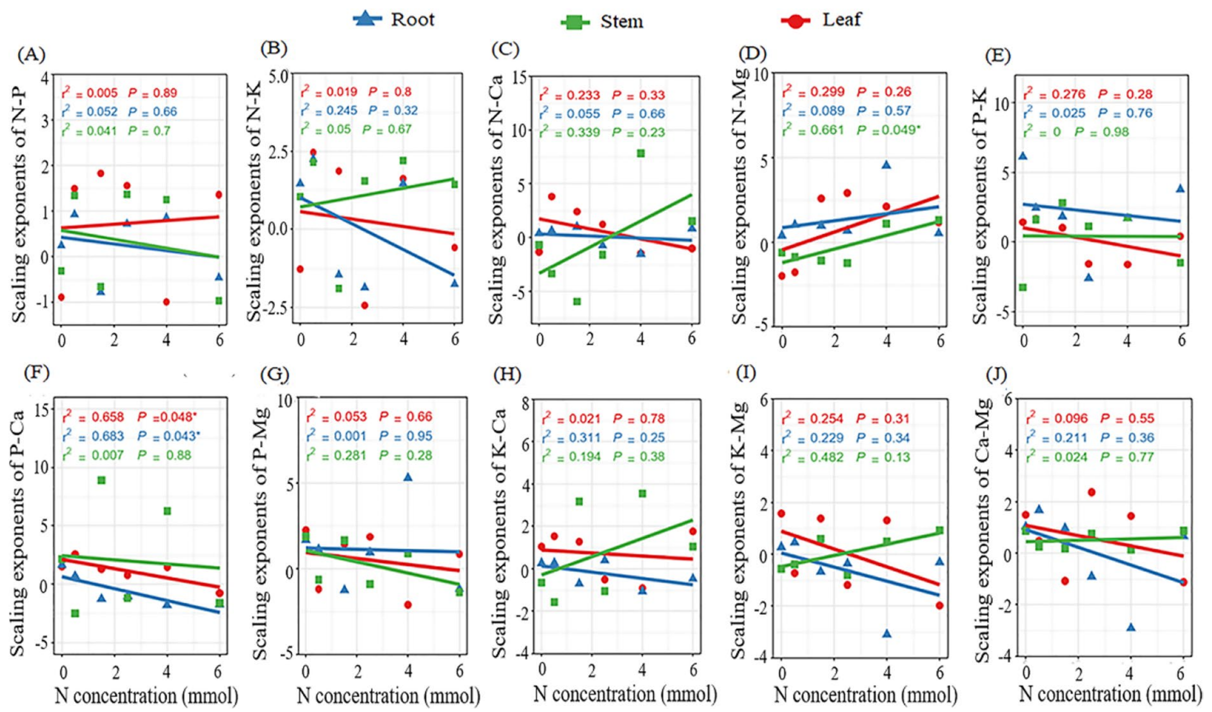

Fig. 3 Scaling exponents among macronutrients to increasing N fertilization in different vegetative organs of hickory saplings ($n = 6$). Root, stem and leaf are represented with blue, green and red circles, respectively. *Adjacent to the p-value indicates a significant change in the scaling exponent from N fertilization

Variable allometric relationships between multinutrients in different organs

As roots, stems, and leaves are the metabolically active organs of plants, they are sensitive to and indicative of environmental change (Walter and Schurr 2005; Schreeg et al. 2014). From the allometric relationships detected for each pair of nutrients, we found the relative accumulation rate of nutrients differed in organs under N fertilization. In leaves, the relative acquisition rate of nutrients are ranked K < Ca < P < Mg < N for macronutrients, and Mn < Zn < Fe for micronutrients. In roots and stems, the relative acquisition rate of macronutrients were observed in the following order: K < P < Ca < N. Mg was not ranked due to its non-significant allometric relationship with N, P, K and Ca, but the SMA showed Mg accumulation trending at a rate that even exceeds N. For micronutrients, both roots and stems showed an accumulation of Fe < Mn < Zn. Therefore, organ-dependent differences in multinutrients allometric relationships were evident under N fertilization.

Leaves are specialists in photosynthesis and transpiration, in which N, Mg and P all play essential roles. In particular, N is a core component of many plant structures and participates in many metabolic processes including cell devision, photosynthesis and transpiration etc., P is involved in the formation of photosynthetic membrane, and Mg is a constituent element of chlorophyll (Liu et al. 2021; Lu et al. 2012; He et al. 2016; Kocheva et al. 2020). Fe is also essential for the synthesis of chlorophyll and a deficiency of Fe will cause interveinal chlorosis of new leaves (Trapet et al. 2020). Thus, leaves may need to acquire more N, Mg, and P than either Ca or K and more Fe than Zn, even though Mn is an essential element of the metalloenzyme cluster of the oxygen-evolving complex in photosystem II, leaves did not acquire more Mn than Fe or Zn, as presented in this study. Roots anchor the plant to the ground and absorb minerals and water from soil, while stems

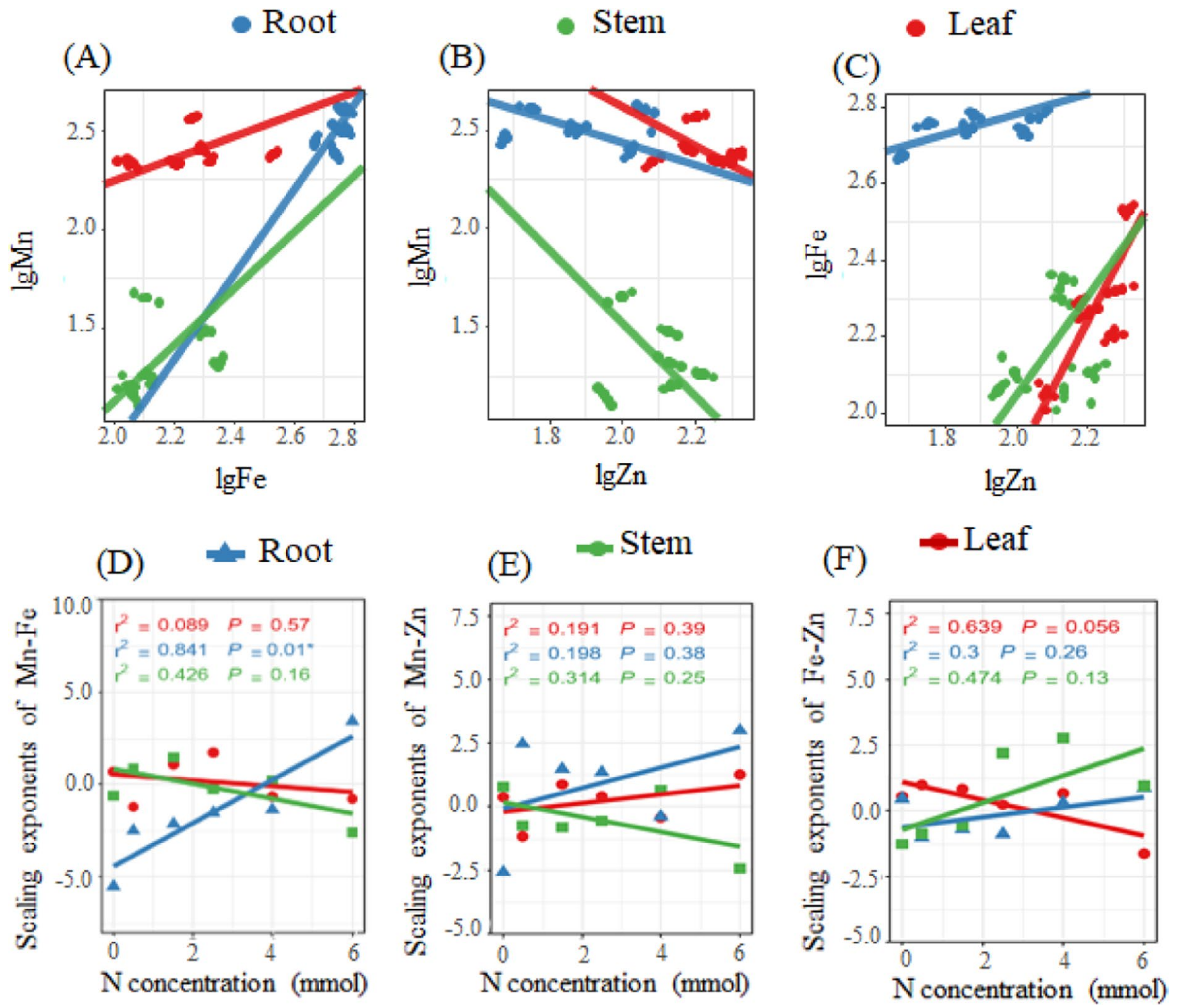

Fig. 4 Allometric relationships among micronutrients in different vegetative organs of hickory saplings using a standardized major axis (SMA) method and their scaling exponents to increasing N fertilization ($n = 6$). Root, stem and leaf are represented with blue, green and red circles, respectively. *Adjacent to the p-value indicates a significant change in the scaling exponent under N fertilization. Note that graph axes are log10 scaled

carry them to all parts of the plants. These differentiated primary functions result in distinct nutrient accumulation strategies between organs (Martínez-Vilalta et al. 2002; Zhou et al. 2020; Tiziani et al. 2020a, b). In roots and stems, the absorption rate of Ca and Zn were greater than other nutrients, highlighting their role in plant acclimatation to N fertilization. For example, Ca is important to maintain the rigidity of cell walls in plants and activates specific enzymes that coordinate certain growth activities, but Ca is not mobile from leaves to plant stems, and is mainly absorbed by roots (Tang and Luan 2017; Kudla et al. 2018). Consequently, roots accumulated the most abundant Ca in this study. Zn is a critical component of proteins, acting as a functional, structural, or regulatory cofactor of a large number of enzymes (Andrejic et al. 2018). Zn deficiency would induce the inhibition of photosynthesis and would also impact gene expression (Kaur and Garg 2021). Therefore, plants prioritize access to Ca and Zn under successive N fertilization.

Contrary to the allometric relationships detected within macronutrients or within micronutrients, in this study, macronutrients generally showed

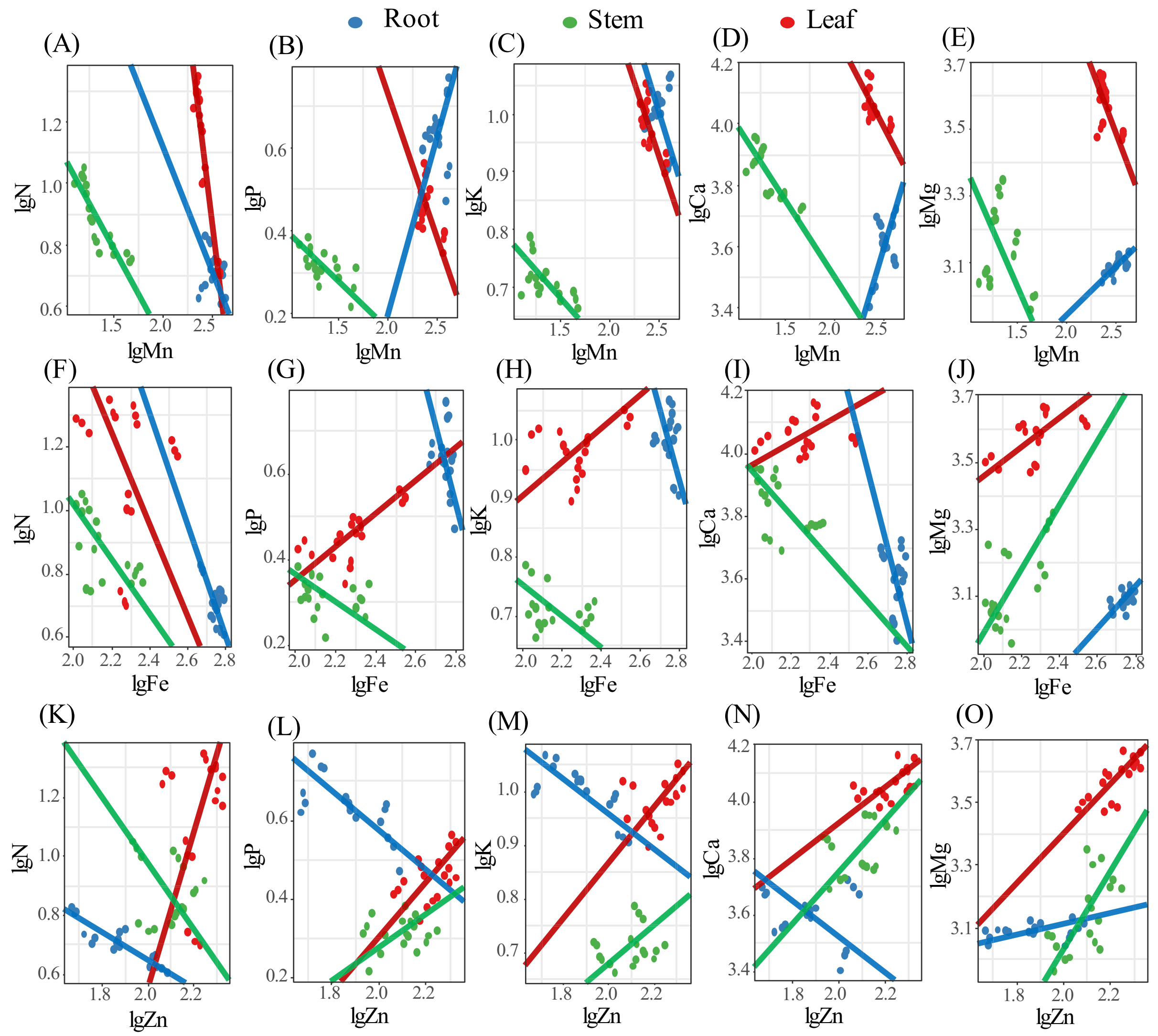

Fig. 5 Allometric relationships between macro- and micronutrients in different vegetative organs of hickory saplings using a standardized major axis (SMA) method ($n=6$). Root, stem and leaf are represented with blue, green and red circles, respectively. Allometric scaling exponent equals to slope of the regression line. Note that graph axes are log10 scaled

negative allometric relationships with micronutrients across vegetative organs. For example, both leaves and stems significantly decreased the accumulation rate of N, P, K, and Ca when Mn acquisition was increased. With increased Zn acquisition the accumulation of P, Ca, and Mg in leaves and N, P, and K in roots was remarkably reduced. These results reveal the trade-offs between macro- and micronutrient investment in hickory saplings under N addition and are generally consistent with other empirical studies (Elser et al. 2010; Ågren and Weih 2012; Mo et al. 2015; Sardans et al. 2017). The trade-off in nutrient investment benefits trees by providing an adaptive capacity to respond to external stress. For example, N fertilization-induced excessive metal elements which not only have a toxic effect on plants, but also reduces the reabsorption efficiency of other nutrients. Trade-offs between nutrients may mitigate these effects (Poschenrieder et al. 2008; Wang et al. 2014). Although some studies report the nutritious response of leaves and fine roots to N fertilization,

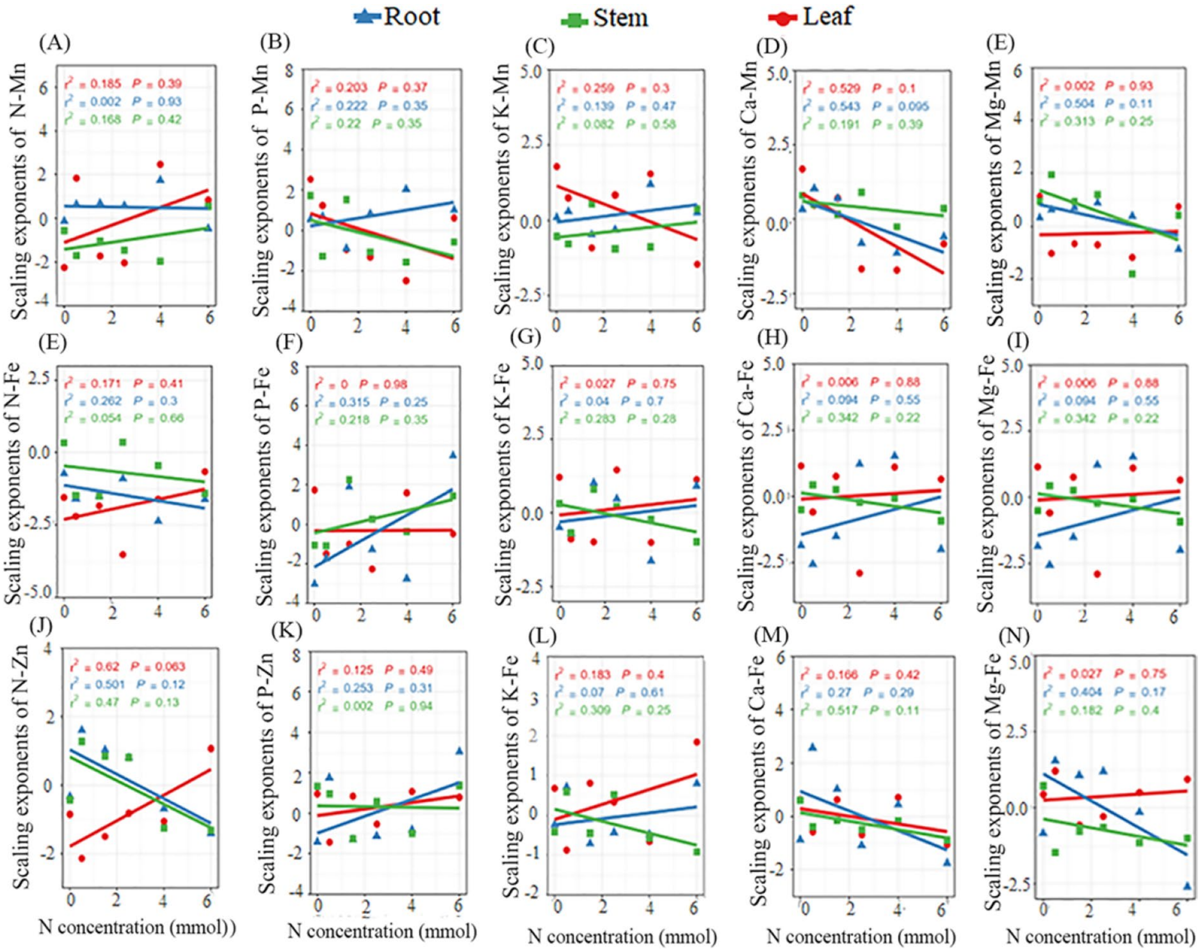

Fig. 6 Scaling exponents between macro- and micronutrients to increasing N fertilization in different vegetative organs of hickory saplings ($n=6$). Root, stem and leaf are represented with blue, green and red circles, respectively. *Adjacent to the p-value indicates a significant change in the scaling exponent under N fertilization

our study found that the scaling relationships between macronutrients and micronutrients were more significantly affected by N fertilization in leaves, while those in stems and roots were less responsive (Kou et al. 2017). This coupling existed in the scaling relationships of different nutrients and is helpful for providing a more holistic understanding of macro- and micronutrient homeostasis in tree species in response to N fertilization.

Differential response of multinutrient allometric relationships to N fertilization

Our results suggest that increasing levels of N fertilization affect organ-level scaling exponents between multinutrients, including leaf N-P, but overall trends were rarely significant. Only 4 (out of 84) pairs of scaling exponents significantly increased or decreased by N enrichment (i.e., stem N-Mg, root P-Ca, leaf P-Ca and root Mn-Fe). This contrasts prior studies indicating that the scaling exponent of leaf N-P is a uniform constant of 0.667 across major life-forms and biomes (Yan et al. 2018). In this study, N-P the scaling exponent fluctuated greatly with increasing N fertilization. This difference likely arises from differences in sample sizes, experimental design, and tree species identity. Generally, a larger sample size benefits more constant scaling exponents, but may also conceal the importance of species specificity (Reich et al. 2010). Our results are based on a short-term hydroponics experiment while most previous studies are reporting results from long-term field experiments (Tian et al. 2018). Further, species identity and maturity (i.e., coniferous vs. broadleaf or sapling vs. mature trees) also differentiate the response of nutrient scaling relationships to N fertilization. We experimentally controlled two-year old Chinese hickory saplings to N addition, while other study results were based on mature trees. We, therefore, saw greater variation in the scaling exponents of our study because of the lower internal stability and resistance of younger trees (Ostertag 2010; Kou et al. 2018; Tian et al. 2018). Besides leaf N-P, we also observed obvious changes in scaling exponents between other nutrients, but currently, there are few similar studies to make our results more comparable. Thus further research on multinutrient allometric relationships that are not limited to N/P is warranted.

Besides the above insignificant changes of scaling exponents, we also found that increasing N fertilization significantly elevated the scaling exponents of stem N-Mg and root Mn-Fe, but reduced root and leaf P-Ca. This indicates that N fertilization influences utilization preference of some nutrients. Specifically, increasing N inputs promotes Ca absorption efficiency over P in leaves and roots. It also stimulates N accumulation rates in stems for acclimation to N addition by ensuring stem growth and nutrient balance are maintained within the hickory saplings. For example, N accumulation in stems increases the structural lignin strength and height of saplings when N fertilization is consistently added (Luo et al. 2019). Besides, more Ca was captured by the roots and transported from the bottom up to leaves as sapling growth is accelerated by N fertilization. This is a result from the fundamental role of Ca in the structuring cell walls and modulating the activity of biological enzymes (Hoang et al. 2020; Kudla et al. 2018). To sum up, N fertilization affects the utilization preference of some nutrients, but the overall utilization strategies of multinutrients were not significantly impacted. Hickory saplings show relatively stable nutrient homeostasis under excessive N input. Mechanisms underlying the variability of nutrient scaling relationship to N fertilization could be intricate, here we proposed the Nutrient Availability-Competition Hypothesis. That is, the competition for N among hickory sapling could be alleviated by consistent N fertilization, but the competition for other nutrients, such as P and Ca, may simultaneously be exacerbated (Wang et al. 2016; Yan et al. 2017). Although we used identical hickory saplings in our experiment, small differences in competition capacity among individuals can not be ignored when considering organ-dependent nutrient concentrations (Sun et al. 2016). Thus, the N fertilization-induced competitive differentiation could be an important explanation for the variation in multinutrients allometric relationships.

Conclusion

In this study, we found more correlational relationships of multinutrients in leaves, but least in roots. We also detected prevalent nutrient allometric relationships between multinutrients, showing organ-dependent nutrient accumulation priorities. For example, leaves acquired more N and Mg than other macronutrients, and more Fe than other micronutrients under the increasing N fertilization. These changes reflect nutrient-level acclimatation of hickory saplings to increasing N availability. Macronutrients generally displayed negative allometric relationships with micronutrients in our results. For instance, increased Mn acquisition significantly decreased the accumulation rate of N, P, K, and Ca in both leaves and stems. This finding reveals tradeoffs in macro- and micronutrients investment that benefit hickory saplings reducing excessive N fertilization stress by developing adaptive functional traits. However, we failed to find any prevailing alterations between scaling exponents of multiple nutrients at the organ-level. In contrast, only 4 out of 84 pairs of scaling exponents were significantly affected by the successive input of N fertilization. This indicates that N fertilization influences the utilization preference of some nutrients, but the overall utilization strategies of multinutrients were not significantly impacted. Hickory saplings show a relatively stable nutrient homeostasis in response to excessive N. Consequently, we proposed the Nutrient Availability-Competition Hypothesis to explain the underlying mechanism of the variation in multinutrient allometric relationships. These results will be helpful to broaden our knowledge of organ-level allometric relationship between multinutrients and may offer new insights to the adaptation of plant functional traits to N fertilization at broader scales.

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Author contributions H.X. carried out the fieldwork and laboratory analysis, prepared figures, and wrote the manuscript. H. X., X. L., D. L and L.S. P. K. revised the manuscript, S. J. contributed substantially to the study design and supervised the field and laboratory personnel.

Declarations

Conflict of interest The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of the funding agencies and supporting institutions. The authors declare that they have no conflict of interest.

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