The Rare-earth Metallome of Pecan and Other Carya

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ABSTRACT. We report the composition of the rare-earth (REE) metallome component of the foliar ionomes of pecan (Carya illinoinensis) and other North American Carya and how accumulation of specific REEs relate to ploidy level and to accumulation of essential divalent nutrient elements. REE accumulation within the foliar ionomes of 12 Carya species, growing on a common site and soil, indicates that REEs accumulate according to the Oddo-Harkins rule with Ce, La, Nd, and Y (Ce > La > Nd > Y) being the dominant REEs with accumulated concentration typically being La > Ce > Nd > Y > Gd > Pr > Sm > Dy > Er > Yb > Ho > Tb > Tm > Se > La. Carya species quantitatively differ in accumulation of REEs with all but C. aquatica accumulating at much greater concentrations than non-Carya tree species and with tetraploid Carya accumulating to approximately twice the concentration as diploid Carya. Carya tomentosa was an especially heavy accumulator of REEs at 859 µg g⁻¹ dry weight, whereas C. aquatica was especially light at 84 µg g⁻¹. Accumulation of REEs was such that any one element within this elemental class was tightly linked (generally r 0.94, but 0.81 for Ce) to all others. Accumulation of REEs is negatively correlated with Ca accumulation and positively correlated with Mn and Cu accumulation in diploid Carya. In tetraploid Carya, accumulated Mg, Ca, and Fe is positively associated with foliar concentration of REEs. Total concentration of REEs in pecan's foliar ionome was 190 µg g⁻¹, about equivalent to that of Mn. Circumstantial evidence suggests that one or more of the physiochemically similar REEs increases physiological plasticity and subsequent adaptive fitness to certain Carya species, especially tetraploids. Because all tetraploid Carya are high REE accumulators and are native to more xeric habitats than diploids, which typically occupy mesic habitats, it appears that REEs might play a role in Carya speciation and adaptation to certain site-limiting environmental stresses. REEs appear to play an unknown metabolic/physiological role in pecan and most Carya species, especially tetraploids; thus, their nutritional physiology merits further investigation.

Pecan and certain Carya species accumulate rare-earth elements [i.e., lanthanides (Lns) and Group III elements of the Periodic Table] as components of foliar ionomes [i.e., all low-molecular-weight molecules and ions present within cells, tissues, organs, or individuals] plus all other elemental forms (Salt et al., 2008)] (Robinson, 1943; Robinson and Edgington, 1945; Robinson et al., 1938, 1958). For example, Carya tomentosa (mockernut hickory) reportedly hyperaccumulates REEs in foliage (Robinson et al., 1938) up to 1350 µg g⁻¹, or 23- to 47-fold greater than most non-Carya tree species on the same forest site (Thomas, 1975). It is unknown whether REEs function in either beneficial or essential roles as components of the metabolomes (i.e., beneficial and essential elements used in metabolic and physiological processes) of pecan or other Carya species or even for any plant species. Thus, composition of the biological periodic table [i.e., elements playing a positive role in life processes (Seravalli and Ragsdale, 2010)] for these species is unknown for elements potentially required at pico- or nanoconcentrations and for “beneficial” nutrient elements. Such elements might function as activation cofactors for enzyme isoforms involved in metabolic fine-tuning in association with tree stress or for imparting the metabolic and physiological plasticity needed for growth in certain environments. Many enzymes contain metals at their catalytic centers (Sun and Chai, 2010) with 47% or more of plant enzymes requiring metals for activity and with metalloenzymes occurring in all Enzyme Commission classes [i.e., ligases (59%), oxidoreductases (44%), transferases (40%), hydrolases (39%), lyases (36%), and isomerases (36%)] (Andreini et al., 2008). It is presently unknown whether any REE functions in a specific biological role in crop plants not otherwise satisfied by known essential nutrient elements (Brown et al., 1990; Hu et al., 2004).

The REEs represent the largest group of elements in the Periodic Table and have potential for yet undetermined essential or beneficial roles in metabolism and physiology. This is because of their quantitative abundance, especially in certain horticultural taxa, and physiochemical similarities to several essential nutrient elements [e.g., Mg, Mn, Fe, and Zn, and especially to that of Ca (Bulman, 2003; Evans, 1983; Franklin, 2001; Lim and Franklin, 2004; Wybourne, 2004)]. It is possible that one or more REEs are important to certain metalloenzyme, or protein isoforms, and are heretofore-overlooked components of the metabolome. Thus, possibly explaining reports of positive REE-based fertilizer or feed supplement effects on many plant crops (Hu et al., 2004; Pang et al., 2002) and animals (He et al., 2001, 2003, 2009; Kraatz et al., 2006; Lei and Xueying, 1997; Weilin et al., 2006).
The REEs are accessible to plants through release into the soil solution from primary (heavy minerals, phosphates, silicates, titanates, fluorides, and carbonates) and secondary (clays, Fe-Mn oxides and carbonates) minerals. The negatively charged organic matter within topsoils is typically REE-enriched through plant recycling, because positively charged REEs readily bind to negatively charged organic matter (Laveuf and Cornu, 2009). Depending on the specific REE, soil abundance is usually greater than that of many essential trace elements and can range from slightly above that of Co or Sn to levels similar to that of Pb and Cd; thus, REEs are rarely rare in soils (Brown et al., 1990). The fact that pecan is typically cultivated in soil environments substantially different from that of the relatively REE-rich humus and topsoils typical of its native habitats raises the possibility that trees within commercial orchards might suffer from chemical stresses linked to a deficiency of one or more REEs. The present study 1) qualitatively and quantitatively characterizes the REE metallomes of the foliar ionome of pecan and related North American Carya species; 2) documents the influence of tetraploidy on the REE metallome; 3) establishes the interrelations of North American Carya taxa based on the REE metallome; 4) documents that Carya species are substantial accumulators of REEs; and 5) postulates a biological role in Carya for REEs as pertaining to isofoms of certain metalloenzymes requiring hard Lewis acids for activity, for cellular signaling, and for enhancing tolerance to drought stress.

**Materials and Methods**

This study presents comprehensive quantitative and qualitative characteristics of the REEs metallome {i.e., scandium (Sc), yttrium (Y), and the Lns [lanthanum (La), cerium (Ce), praseodymium (Pr), neodymium (Nd), samarium (Sm), europium (Eu), gadolinium (Gd), terbium (Tb), dysprosium (Dy), holmium (Ho), erbium (Er), thulium (Tm), ytterbium (Yb), and lutetium (Lu)]} of pecan and North American Carya. Promethium (Pm) is excluded because natural abundance is trivial and all isotopes are short-lived and radioactive. Physiochemical similarities enable classification into four subgroups—i.e., “Group II REES” [G-II REES (Sc, Y)], “light REES” [LREES (La, Ce, Pr, and Nd)], “intermediate REES” [IREES (Sm, Eu, Gd, Tb, and Dy)], and “heavy REES” [HREES (Ho, Er, Tm, Yb, and Lu)] (Laveuf and Cornu, 2009).

**Experimental site and plant material.** Seed from 12 Carya species native to the United States were collected from each of three wild mother trees growing in native habitats within each species’ natural range. Mother trees were generally separated by ≥100 m or greater. Seed were germinated and grown in pots for 2 years, after which two seedlings were randomly selected from each of three maternal parents of each species and planted in a common field plot established on a well-drained Norfolk loamy fine sand [Ng (fine-loamy, Kaolinitic, thermic Typic Kandiudult soil; a common soil in the Southern Coastal Plain, Atlantic Coast Flatwoods, and Tidewater Area of the United States)] to give six replications of any one species. The planting site was at Byron, GA, a humid climatic zone (lat. 32°39’54” N, long. 83°44’31” W; 155 m elevation) with a frost-free growing period of ≈280 d and an annual precipitation of ≈130 cm. Test trees were grown at 10 × 10-m spacing without irrigation, fertilization, or pesticides (except for contact herbicides to control weeds). The site had not received fertilizers for ≥20 years before tree establishment. A single 2-year-old seedling of each species was randomly established within a block containing 12 Carya species with the pattern replicated to give six blocks (i.e., 72 trees). Trees were 22 years old at sampling.

Sampled Carya species represent both Apocarya and Carya sections of subtribe Caryinae of the tribe Juglandeae (Juglandaceae) (Manos and Stone, 2001) (Table 1). Species differ in not only morphology and habitat, but also in ploidy (i.e., 2n = 32 vs. 64) with Apocarya section species being diploid (100% of species) and Carya section species being either diploid (40% of taxa) or tetraploid (60% of taxa). Species are grouped by section based on morphology with those within the Carya section being shellbark hickory [C. laciniosa (2n = 32)], southern shagbark hickory [C. carolinae-septentrionalis (2n = 32)], sand hickory [C. ovata (2n = 32)], pecan [C. pallida (2n = 64)], mockernut hickory [C. tomentosa (2n = 64)], pignut hickory [C. glabra (2n = 64)], black hickory [C. texana (2n = 64)], and scrub hickory [C. floridana (2n = 64)]. Apocarya section species are pecan [C. illinoinsensis (2n = 32)], water hickory [C. aquatica (2n = 32)], and bitternut hickory [C. cordiformis (2n = 32)]. Species not included were mexican hickory [C. palmeri (found only in Mexico)] of the Apocarya section and red hickory [C. ovalis] of the Carya section. Other excluded species are the Asian section Sinocarya (C. cathayensis, C. dabieshanensis, C. hunanensis, C. kweichowensis, C. poilanei, and C. tonkinensis).

The hyperaccumulation and phytomining potential of pecan and other Carya species was assessed by comparing accumulated elements within foliar organs (i.e., leaves) from several non-Carya species growing nearby on the same soil type. These

| Carya species | Common name          | Ricorea sections | Ploidy (2n) | Seed location U.S. state (county/parish) |
|--------------|----------------------|-----------------|------------|----------------------------------------|
| C. aquatica  | Water hickory        | Apocarya        | 32         | Louisiana (Caddo)                      |
| C. cordiformis | Bitternut hickory   | Apocarya        | 32         | Louisiana (DeSoto)                     |
| C. floridana | Scrub hickory        | Carya           | 64         | Florida (Marion)                       |
| C. glabra    | Pignut hickory       | Carya           | 64         | Georgia (Crawford)                     |
| C. illinoinsensis | Pecan hickory    | Apocarya        | 32         | Mississippi (Washington)               |
| C. laciniosa | Shellbark hickory    | Carya           | 32         | Kentucky (Union)                       |
| C. myristiciformis | Nutmeg hickory  | Carya           | 32         | Louisiana (Caddo)                      |
| C. ovata     | Shagbark hickory     | Carya           | 32         | Kentucky (Crittenden)                  |
| C. pallida   | Sand hickory         | Carya           | 64         | Georgia (Bibb)                         |
| C. carolinae-septentrionalis | Southern shagbark hickory | Carya           | 32         | Georgia (Jones)                        |
| C. texana    | Black hickory        | Carya           | 64         | Louisiana (DeSoto)                     |
| C. tomentosa | Mockernut hickory    | Carya           | 64         | Louisiana (Tangipahou)                 |
are slippery elm (Ulmus rubra), red maple (Acer rubrum),
american persimmon (Diospyros virginiana), southern red oak
(Quercus falcata), sassafras (Sassafras albidum), and sweet-
gum (Liquidambar styraciflua), thus representing different
families (i.e., Ulmaceae, Ebenaceae, Aceraceae, Lauraceae,
Rosaceae, and Fagaceae) than that of the Carya species (i.e.,
Juglandaceae).

**RARE-EARTH ELEMENT METALLOME ANALYSIS.** A snapshot of
the foliar ionomes and metallomes was derived by randomly
sampling mature foliage from 10 long vegetative shoots col-
lected around the lower sun-exposed canopy of individual trees.
Foliar sampling was from the middle leaflets of middle com-
 pound leaves of each sampled shoot as is the standard practice
for leaf analysis sampling in commercial pecan orchards.
Sampling was taken during early August within the 15 July to
15 Aug. window as recommended by extension specialists for
tissue sampling to assess tree nutrient element health. Foliage
from non-Carya species was sampled and processed as described
for *Carya* species; midshoot simple leaves were sampled if the
vegetative shoots did not possess compound leaves. Six trees of
each non-pecan species were sampled from wild trees growing
nearby on non-fertilized Norfolk loamy fine sand soil. To reduce
the probability that detected REEs were surface contaminates
from dust, sampled leaflets were combined for individual trees
and rinsed with agitation 30 s in 2% nitric acid and triple rinsed
with agitation in deionized water. Samples were then dried to
a constant weight at 55 °C, ground in a Wiley mill, and 500 mg
digested in 10 mL of 70% ultralow trace element grade nitric
acid using a MarsXpress carousel in a Mars-5 microwave oven
(CEM Corp., Matthews, NC). Microwave digestion was as fol-
sows: temperature was ramped from room temperature to
210 °C over 12 min and held at 210 °C for 13 min. Cooled
digested samples were filtered, brought to 20 mL (by weight;
using 2% nitric acid) in a 50-mL polypropylene centrifuge tube,
and 0.10 to 1.00 mL (or 0.100 mL added to a 15-mL poly-
propylene disposable centrifuge tube and brought up to 14 mL
using 2% ultrapure nitric acid made with greater than 18 MΩ
deionized water.

**INDUCTIVELY COUPLED PLASMA QUADROPOLE SPECTROMETER
ANALYSIS.** The concentration of all REEs plus other elements
similar to REEs (i.e., Ca, Mg, Mn, Fe, Cu, and Zn) in foliage
was determined using an inductively coupled plasma quadrupole
spectrometer SCIEX ELAN-9000; PerkinElmer, Concord,
Ontario, Canada). Quantitative analysis was facilitated by
various similar-mass internal standards (Li, Ge, In, Ce, W, or Ba)
(daturing on the elements being analyzed) and by external standards using multielement standard solutions (Perki-
Elmer Multielement Calibration Standard Sets) diluted to cover
three to four orders of concentration magnitude.

**STATISTICAL ANALYSIS.** Metal analysis was based on two
offspring from each of three mother trees of 12 *Carya* species.
The experiment had a randomized complete block design with
single tree pots for each of 12 species per block and six replicates
( *n* = 72). Analysis of variance (*P* ≤ 0.05) for species and ploidy
treatments was determined for foliar elemental parameters.
Elemental accumulation relationships were identified using multivari-
ate pairwise correlation analysis. Concentration means for each
species were analyzed through multivariate hierarchical clus-
tering using the Ward’s minimum variance method for defining
distances between clusters to yield dendrograms illustrating
species and relationships within ploidy classes.

**Results**

**RARE-EARTH METALLOMES.** The elemental composition of the
foliar REE metallome of North American *Carya* did not
qualitatively differ among species; however, quantitative differ-
cences were substantial with individual elemental concentrations
differing severalfold (Tables 2 and 3). The REEs of highest
concentration varied among species but were generally Ce > La
> Nd > Y with all other REEs present at much lower concentrations
(Tables 2 and 3; Fig. 1). Differences in the relative concentration
of individual REEs are too numerous to discuss herein but are
listed in Tables 2 and 3 for future baseline reference.

It is noteworthy that *C. tomentosa* contains relatively high
concentrations of Ce (326 μg·g⁻¹), La (144 μg·g⁻¹), Nd (136
μg·g⁻¹), and total REEs (859 μg·g⁻¹). The concentrations of
these three dominating REEs were 83%, 45%, and 37%,
respectively, of that found earlier by Thomas (1975). Addi-
tionally, these three Lns comprise 71% of all REEs detected,
which compares with 80% found earlier in *C. tomentosa*. Much
of this discrepancy is likely the result of differences in soil REE

### Table 2. Relative prominence of the light rare-earths in foliage of several North American *Carya* species.

| *Carya* species | Sc (ng·g⁻¹) | Y (μg·g⁻¹) | La (μg·g⁻¹) | Ce (μg·g⁻¹) | Pr (μg·g⁻¹) | Nd (μg·g⁻¹) | Sm (μg·g⁻¹) | Eu (μg·g⁻¹) |
|----------------|------------|------------|------------|------------|------------|------------|------------|------------|
| *C. aquatica*  | 184.7 ab   | 16.4 d     | 10.8 d     | 27.0 e     | 2.9 d      | 13.2 d     | 2.7 c      | 0.5 e c    |
| *C. cordiformis*| 66.8 ab    | 84.6 abc   | 108.6 ab   | 276.0 ab   | 26.5 ab    | 105.8 ab   | 18.9 ab    | 3.8 ab     |
| *C. floridana* | 60.7 ab    | 85.2 abc   | 85.3 ab–d  | 164.2 b–e  | 23.9 bc    | 89.9 ab    | 18.4 ab    | 3.4 abc    |
| *C. glabra*    | 89.0 ab    | 120.5 a    | 145.7 a    | 240.4 ab   | 33.8 a     | 133.6 a    | 23.9 a     | 4.9 a      |
| *C. illinoensis*| 49.0 ab    | 35.4 cd    | 39.0 bcd   | 38.0 e     | 9.2 cd     | 36.8 cd    | 6.3 c      | 1.3 cde    |
| *C. laciniosa* | 78.0 ab    | 88.0 abc   | 93.2 abc   | 135.7 cde  | 23.0 abc   | 91.7 abc   | 17.7 ab    | 3.3 a–d    |
| *C. myristiciforis* | 140.0 ab | 25.3 cd    | 27.8 cd    | 55.5 de    | 6.5 d      | 26.9 cd    | 4.9 c      | 1.0 de     |
| *C. ovata*     | 247.7 a    | 68.6 a–d   | 96.8 ab    | 126.6 cd   | 23.1 abc   | 91.8 ab    | 19.3 a     | 3.3 a–d    |
| *C. pallida*   | 103.2 ab   | 96.4 ab    | 120.1 a    | 153.3 b–e  | 28.0 ab    | 108.9 ab   | 19.3 ab    | 3.9 ab     |
| *C. septentrionalis* | 26.7 b | 97.8 ab    | 100.2 abc  | 180.4 bcd  | 29.4 ab    | 120.4 ab   | 22.9 a     | 4.6 a      |
| *C. texana*    | 9.8 b      | 54.2 bcd   | 66.8 a–d   | 84.5 de    | 15.5 bcd   | 62.0 bcd   | 10.8 bc    | 2.2 b–e    |
| *C. tomentosa* | 229.2 ab   | 106.6 ab   | 144.5 a    | 325.5 a    | 34.4 a     | 136.4 a    | 24.3 a     | 4.8 a      |

*Means within a column and followed by different letters are statistically different at *P* ≤ 0.05 using Tukey-Kramer honestly significant difference test.
*Carya carolinae-septentrionalis* is referred to herein as *C. septentrionalis.*
composition and bioavailability and tree genotype. By comparison, the REE concentration of *C. aquatica* was especially low at 84 μg·g⁻¹ or approximately one-tenth that of *C. tomentosa*. The relative concentration of REEs in pecan, a diploid, is La > Ce > Nd > Y > Gd > Pr > Sm > Dy > Er > Yb > Ho > Tb > Tm > Sc > Lu. Several REEs (Y, La, Ce, Nd) are present in foliage at approximately the same concentration as are Fe, Zn, and B; and others (Pr, Sm, Gd, Dy) are present at approximately the same concentration as Cu and still others (Tb, Eu, Ho, Er, Tm, Yb, Lu) approximately the same concentration as Ni and Mo. When viewed collectively, the concentration of all REEs in pecan is ≈190 μg·g⁻¹ dry weight, which is similar to that of Mn in many orchard trees and ≈20% as the critical level of phosphorus and sulfur. Total REE concentration in pecan is only ≈4% to 13% as high as that of Ca and Mg, respectively, when these macronutrients are near the concentration that triggers visual deficiency symptoms.

Accumulation of the individual lanthanide constituents of the *Carya* foliar REE metallome is highly correlated [correlation coefficient ($r$)] among constituent members (data not included) with $r$ ranging from 0.93–0.99 ($P < 0.001$) for all Ln pairs, except for Ce, which ranged from 0.77–0.94 ($P < 0.001$) depending on the Ln. This is true for pecan as well as for diploid and tetraploid *Carya*. Such tight linkage suggests that Ln uptake mechanism and transporters in *Carya* taxa possess little or no specificity for the individual Lns, except with the possible exception of Ce. This might be because Ce typically exists as a tetravalent cation in soil solutions, whereas the other REEs typically exist as trivalent cations.

The accumulation of REEs in *Carya* is such that those with even atomic numbers (Z) are present at greater concentration than that of the two adjacent elements with odd Z (Fig. 1). This pattern is consistent with that found by Watanabe et al. (2007) for Ln composition of several orders of plants. Thus, relative REE accumulation patterns in *Carya* are consistent with that observed for other plant species being consistent with the Oddo-Harkins rule, which holds that elements with an even Z are more common than elements with an odd Z. This is because elements with an even proton number are balanced in proton spin and less likely to capture an additional proton during formative processes than odd-numbered elements possessing an unpaired proton.

When tetraploid and diploid *Carya* classes are compared against several non-*Carya* (from non-Juglandaceae) species, it is apparent that both *Carya* ploidy classes accumulate REEs at much greater concentrations than do many other species when grown under similar conditions (Table 4). Tetraploid *Carya* as a group possessed much higher REE concentrations than did diploid *Carya* but with relative concentrations among REEs being equivalent for taxa within each of the two ploidy levels. Accumulation of G-II REEs, LREEs, IREEs and HREEs, Lns, and total REEs by tetraploids was ≈1.7- to 1.8-fold greater than
that by diploids and 32- to 38-fold greater than by non-Carya species (Table 4). In terms of specific REE elements, tetraploids accumulated more Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, and Lu than diploids; however, diploids accumulated more Sc, the lightest of the REEs, than tetraploids. Both tetraploids and diploids accumulated all REEs at a greater concentration than did non-Carya species found growing in the same or similar habitats.

If REEs act as analogs to certain essential divalent metals [M$^{2+}$ (e.g., Ca, Mg, Fe, Cu, and Zn)] of the Carya metabolome, then the M$^{2+}$/REE ratio likely varies among Carya species and relatively strong correlations with accumulation of these other metals. The M$^{2+}$/REE ratio of key essential metals within the Carya metabolome indeed varies depending whether the foliar ionome originated from tetraploid or diploid Carya or from non-Carya species. In the case of essential nutrient elements that function in planta as hard Lewis acids (i.e., Mg, Ca, and Zn) these ratios were very high for non-Carya species (Table 5) but relatively low for diploid Carya and especially low for tetraploid Carya (i.e., Mg/G-II REEs, Mg/LREEs, Mg/IREEs, Mg/HREEs, Mg/Total REEs, Ca/G-II REEs, Ca/LREEs, Ca/IREEs, Ca/HREEs, Ca/Total REEs, Zn/G-II REEs, Zn/LREEs, Zn/IREEs, and Zn/Total REEs). In the case of the intermediate strength Lewis acids (e.g., Mn, Fe, and Cu), there were no REE ratio differences between tetraploid and diploid Carya; however, almost all ratios were much greater for non-Carya species than for either Carya ploidy class (Table 5). These REE ratio differences tend to support the possibility that one or more REEs might act in planta because limited substitutes for certain processes usually requiring Lewis acids (Mg, Ca, Zn, Mn, Fe, Cu, Co, Ni).

It is noteworthy that for diploid Carya, the accumulated concentration of Mn within the foliar ionome exhibits substantial positive correlation (r = 0.59–0.72; data not shown) with accumulated concentration of elements comprising the various REE classes, whereas there were negative associations with Cu. For the individual REEs, Mn was positively correlated with all REEs (r = 0.43–0.72) as was Co (r = 0.40–0.57), but Cu was negatively correlated (r = -0.45–0.54). For tetraploid Carya, accumulation of Mg (r = 0.24–0.40), Ca (r = 0.24–0.41), and Fe (r = 0.59–0.67) in the foliar ionomes is positively associated with the concentration of various REE classes. The accumulation of Mn in pecan, and diploid Carya, is positively correlated with each of the 14 individual Lns (r = 0.62–0.72). Also, there was no significant relationship between Mn, Cu, Co, Ni, Zn, and Mo and any specific Ln; however, Fe accumulation is positively associated (r = 0.56–0.65) with all Lns. There is also a weak positive correlation between Mg and La (r = 0.45), Ca and La (r = 0.42), Mg and Ce (r = 0.39), and Ca and Ce (r = 0.41) for tetraploid Carya. Thus, the accumulation of all REEs in diploid Carya is positively correlated with Mn and Co but negatively associated with Cu, and there was no correlation of Mg or Ca with any REE. Conversely, in tetraploid Carya, Fe is the only micronutrient correlated with the individual REEs; however, the macronutrients Mg and Ca are positively correlated with La and Ce.

**Cluster analysis of foliar rare-earth metallome.** Hierarchical cluster analysis of the multivariate components of the REE metallome of the foliar Carya ionomes, when the variance within a cluster is minimized (i.e., Ward’s method), produced dendrograms with relatively long stems and small distances within the far branches of the trees, hence identifying likely discrete natural grouping within each of the two ploidy classes (Fig. 2). The SES were small in each dendrogram but slightly larger for the tetraploid population than that of the diploid population, indicating what might be greater variability within the REE metallome components of tetraploid than diploid taxa. The clustering of C. aquatica through chaining to the C. illinoinensis/myristiciformis cluster, while possessing a short stem-associated distance metric, indicates that although the REE metallome of C. aquatica is distinct, it possesses substantial similarity to that of the other two species. Similar close relationships exist between C. carolina-septentrionalis (of section Caryta) and C. cordiformis (of section Apocarya). The REE metabolomes of C. ovata and C. laciniosa are also very similar to each other and also similar to C. carolina-septentrionalis and

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**Table 4. Comparison of rare-earth (REE) metal composition of the foliar ionome of diploid (2n = 32) and tetraploid (2n = 64) Carya species and non-Carya species.**

| Group II REEs (µg g$^{-1}$) | Tetraploid species | Diploid species | Non-Carya species |
|-----------------------------|-------------------|----------------|------------------|
| Sc (ng g$^{-1}$)            | 58.1 b            | 127.7 a        | 13.6 c           |
| Y (µg g$^{-1}$)             | 93.46 a           | 53.0 b         | 2.9 c            |
| La (µg g$^{-1}$)            | 110.4 a           | 62.7 b         | 3.4 c            |
| Ce (µg g$^{-1}$)            | 191.4 a           | 109.8 b        | 4.2 c            |
| Pr (µg g$^{-1}$)            | 27.5 a            | 15.2 b         | 0.9 c            |
| Nd (µg g$^{-1}$)            | 108.5 a           | 61.1 b         | 3.5 c            |
| Sm (µg g$^{-1}$)            | 19.9 a            | 11.6 b         | 0.6 c            |
| Eu (µg g$^{-1}$)            | 4.0 a             | 2.2 b          | 0.2 c            |
| Gd (µg g$^{-1}$)            | 30.1 a            | 17.9 b         | 1.2 c            |
| Tb (µg g$^{-1}$)            | 2.8 a             | 1.6 b          | 0.1 c            |
| Dy (µg g$^{-1}$)            | 16.0 a            | 9.1 b          | 0.5 c            |
| Ho (µg g$^{-1}$)            | 2.9 a             | 1.7 b          | 0.1 c            |
| Er (µg g$^{-1}$)            | 8.3 a             | 4.8 b          | 0.2 c            |
| Tm (ng g$^{-1}$)            | 991.1 a           | 567.0 b        | 27.4 c           |
| Yb (µg g$^{-1}$)            | 62.2 a            | 3.5 b          | 0.2 c            |
| Lu (ng g$^{-1}$)            | 934.6 a           | 526.7 b        | 27.9 c           |

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* Tetraploid species are sand hickory [C. palla (2n = 64)], mockernut hickory [C. tomentosa (2n = 64)], pigment hickory [C. glabra (2n = 64)], black hickory [C. texana (2n = 64)], and scrub hickory [C. floridana (2n = 64)]. Diploid species are shellbark hickory [C. laciniosa (2n = 32)], southern shagbark hickory [C. carolina-septentrionalis (2n = 32)], shagbark hickory [C. ovata (2n = 32)], pignut hickory [C. myristiciformis (2n = 32)], pecan [C. illinoinensis (2n = 32)], water hickory [C. aquatica (2n = 32)], and bittersnut hickory [C. cordiformis (2n = 32)]. Non-Carya species are slippery elm (Ulmus rubra), red maple (Acer rubrum), american persimmon (Diospyros virginiana), southern red oak (Quercus falcata), sassafras (Sassafras albidum), and sweetgum (Liquidambar styraciflua).

* Group II REEs (G-II REEs (Sc, Y)), light REEs (LREEs (La, Ce, Pr, Nd)), intermediate REEs (IREEs (Sm, Eu, Gd, Tb, Dy)), and heavy REEs (HREEs (Ho, Er, Tm, Yb, Lu)).

* Means within the same row (i.e., element or elemental class) followed by different letters are different at P = 0.05 by Tukey-Kramer honestly significant difference.
Table 5. Ratios of different classes of rare-earth elements (REEs) in the foliar ionome of diploid and tetraploid Carya and in non-Carya species to that of chemically similar metabolome metals.1

| Element (units) | Tetraploid species | Diploid species | Non-Carya species |
|----------------|-------------------|----------------|------------------|
| **Metals**     |                   |                |                  |
| Mg (µg g−1)    | 4,521a            | 5,188a         | 4,746a           |
| Ca (µg g−1)    | 17,445a           | 18,962a        | 10,819b          |
| Mn (µg g−1)    | 2,401a            | 1,791b         | 1,291c           |
| Fe (µg g−1)    | 58b               | 82a            | 80a              |
| Cu (µg g−1)    | 9b                | 13a            | 11b              |
| Zn (µg g−1)    | 89a               | 102a           | 15b              |
| Total REEs (µg g−1) | 624a          | 355b           | 18c              |

**Metal/rare-earth ratio**

| Mg/G-II REEs | 58 c | 136 b | 7,710 a |
| Mg/LREEs     | 13 c | 37 b  | 981 a   |
| Mg/IREEs     | 75 c | 192 b | 4,999 a |
| Mg/HREEs     | 1,083 c | 2,668 b | 104,961 a |
| Mg/Total REEs| 9 c  | 24 b  | 711 a   |
| Ca/G-II REEs | 222 c | 510 b  | 17,919 a |
| Ca/LREEs     | 51 c | 133 b | 2,137 a |
| Ca/IREEs     | 285 c | 706 b  | 10,979 a |
| Ca/HREEs     | 1,083 c | 2,668 b | 104,961 a |
| Ca/Total REEs| 35 c | 88 b  | 1,558 a |
| Mn/G-II REEs | 33 b | 43 b  | 2,355 a |
| Mn/LREEs     | 8 b  | 11 b  | 273 a   |
| Mn/IREEs     | 45 b | 60 b  | 1,571 a |
| Mn/HREEs     | 164 b | 223 b | 15,201 a |
| Mn/Total REEs| 5 b  | 7 b   | 202 a   |
| Fe/G-II REEs | 1 b  | 2 b   | 130 a   |
| Fe/LREEs     | 0.2 b | 0.6 b | 16 a    |
| Fe/IREEs     | 1 b  | 3 b   | 84 a    |
| Fe/HREEs     | 4 b  | 12 b  | 789 a   |
| Fe/Total REEs| 0.1 b | 0.4 b | 12 a    |
| Cu/G-II REEs | 0.1 b | 0.4 b | 16 a    |
| Cu/LREEs     | 0.1 b | 0.1 b | 2 a     |
| Cu/IREEs     | 0.2 b | 0.6 b | 11 a    |
| Cu/HREEs     | 0.7 b | 2 b   | 99 a    |
| Cu/Total REEs| 0.1 b | 0.1 b | 2 a     |
| Zn/G-II REEs | 1.2 c | 2.9 b | 24.5 a  |
| Zn/LREEs     | 0.3 c | 0.8 b | 3.1 a   |
| Zn/IREEs     | 1.5 c | 4.1 b | 16.2 a  |
| Zn/HREEs     | 1.3 b | 1.3 b | 146 a   |
| Zn/Total REEs| 0.2 c | 0.5 b | 2.5 a   |

1Tetraploid species are sand hickory [C. pallida (2n = 64)], mockernut hickory [C. tomentosa (2n = 64)], pignut hickory [C. glabra (2n = 64)], black hickory [C. texana (2n = 64)], and scrub hickory [C. floridana (2n = 64)]. Diploid species are shellbark hickory [C. illinoinensis (2n = 32)], southern shagbark hickory [C. cordiformis (2n = 32)], southern shagbark hickory [C. aquatica (2n = 32)], pignut hickory [C. illinoinensis (2n = 32)], and bitternut hickory [C. cordiformis (2n = 32)].

2Group II REEs [G-II REEs (Sc, Y)], light REEs [LREEs (La, Ce, Pr, Nd)], intermediate REEs [IREEs (Sm, Eu, Gd, Tb, Dy)], and heavy REEs [HREEs (Ho, Er, Tm, Yb, Lu)].

3Means within the same row (i.e., element or elemental class or ratio) followed by different letters are different at P = 0.05 by Tukey-Kramer honestly significant difference test.

The REEs metallomes of the five tetraploid taxa clearly differentiates into two distinct groups with C. tomentosa and C. glabra being more similar to each other than to the other three diploid taxa (i.e., C. myristiciformis, C. illinoinensis, and C. aquatica).

The REEs metallomes of these four species possess REE metallomes more similar to each other than to the other three diploid taxa (i.e., C. myristiciformis, C. illinoinensis, and C. aquatica).

**Discussion**

**Potential of a physiological role for rare-earth element in Carya species.** Brown et al. (1990) reviewed possible generalized physiological roles for REEs and concluded they could potentially influence membrane stability and ion transport (especially Ca2+, K+, and Na+), nitrogen fixation, disease resistance, hormonal interactions, sensing, and enzyme activity. The hyperaccumulation documented here provides circumstantial evidence that one or more REEs might theoretically benefit Carya metabolism or physiology. However, hyperaccumulation does not necessarily mean that REEs play a biological role. It is possible that the root system of Carya species, especially that of tetraploids, fails to bind up excessive REEs as effectively as that of diploids or of the roots of non-REE accumulating non-Carya species. If one or more REEs possess biological relevance in Carya, then the ion must pass through cell membranes and enter symplastic space. The present study does not discriminate whether REEs are in symplasmic or apoplasmic space or are bound to cell walls, accumulated in vacuoles, or in free cytoplasmic space. Future research needs to identify their locations if the metabolic or physiological relevance is to be understood.
Many metalloenzymes, especially hydrolytic enzymes, require hard Lewis acids (e.g., Ca, Mg, and Zn) for activation of key elemental bonds requiring cleavage. Because the REEs are hard Lewis acids, have high coordination numbers, exhibit fast ligand exchange rate characteristics, and have relatively inaccessible redox chemistry, they are well suited as potential cofactors in certain metabolically relevant enzymes (Franklin, 2001). They appear to be especially suitable for replacing Ca and certain other alkali-earth and outer transition-metal elements within various enzymatic proteins or for Ca in intracellular cytosolic signaling (Bulman, 2003; White and Broadley, 2003). Lanthanide ions are near ideal Ca analogs and can substitute for Ca in proteins under artificial conditions with minimal structural changes, but binding is likely to be less reversible than Ca$^{2+}$ (Evans, 1983). Thus, if one or more REEs replace Ca at membrane sites, or pass through cellular membranes, then the REE can potentially engage in a host of critical metabolic processes. This raises the possibility of both competitive inhibition of Ca-dependent processes and the existence of Ln-dependent metalloenzyme isoforms and Ca$^{2+}$-like signaling processes in *Carya*. REEs could also act as Ca$^{2+}$ surrogates to enhance crosslinking of membranes, thus stabilizing membranes under acidic soil conditions, where Ca might be limiting, and there is a need to stabilize ion transmembrane transport (White and Broadley, 2003). Additionally, because Lns have high binding affinities to the plasmalemma and other intracellular membranes, they potently influence ion channels and therefore signaling and gene expression (Hagenbeck et al., 2000; Rock and Quatrano, 1996).

If REEs act as Ca (or Mg, Fe, Cu, Zn) analogs to the essential Lewis acid metals of the *Carya* metabolome, then the M$^{2+}$-REEs likely varies among *Carya* species, and there is likely to be strong correlations in element accumulation. The ratio of key essential elements within the *Carya* metabolome to the various classes of REEs indeed varied depending whether the foliar ionome originated from tetraploids or diploids or from non- *Carya* species. These ratios were very high for non- *Carya* species but much lower for diploid *Carya* and especially low for tetraploids (i.e., Mg/G-II REEs, Mg/LREEs, Mg/IEREs, Mg/HREEs, Mg/Total REEs, Ca/G-II REEs, Ca/LREEs, Ca/IEREs, and Ca/HREEs. Ca/HREEs, Ca/Total REEs, Zn/G-II REEs, Zn/LREEs, Zn/IEREs, and Zn/Total REEs). In the case of certain intermediate-strength Lewis acids (e.g., Mn, Fe, and Cu), there were no M$^{2+}$-REE differences between tetraploids and diploids; however, almost all ratios were much greater for non- *Carya* species than for either *Carya* ploidy class. These ratio differences suggest that one or more REEs might substitute for Mg, Ca, and Zn in tree processes requiring hard Lewis acids and also possibly for Mn, Fe, Co, and Cu in one or more specific processes where an intermediate-strength Lewis acid-requiring process might be satisfactorily substituted by a hard Lewis acid. These possibilities are supported in that REE ionic radii are very similar to that of Ca and several other essential divergent cations depending on the coordination environment in which ions are exposed (Barbalace, 2011; Grimes and Wenman, 2011).

Calcium homeostasis is critical to the effectiveness of stimulus-response systems operating in planta (Sanders et al., 1999). If certain REEs are replacing Ca, or possess similar signaling roles, then there would be need for a similar tight regulation of cytosolic REE concentration. Thus, a measure of cytosolic REE concentration would provide evidence pertaining to whether REEs are likely to be involved in "signaling" with concentrations similar to the of Ca$^{2+}$ ([100–200 nM (Nayyar, 2003)] being evidence of activity. The Lns can replace Mg and Zn in certain proteins and enzymes, and Gd and La act as Ca analogs in Ca-channel studies (Linn and Franklin, 2004), and they also bind with Ca-dependent protein kinases involved in key cellular processes (Plieth, 2005). It seems likely that elements nearly identical to Ca (i.e., the Lns) are involved in life processes, yet no critical roles for Lns, or other REEs, are known in any plant species. This is surprising given that Lns are so similar in ion size, coordination environment, ligand preferences, and Lewis acid activity (giving good hydrolytic activity). Many metals are ideally suited for specific biochemical functions; thus, they are commonly used in life processes. Had the Lns been as abundantly available early on, and over evolutionary time, as what are today the essential hard Lewis acid elements (e.g., Ca$^{2+}$, Mg$^{2+}$, and Zn$^{2+}$), then the Lns might today be obviously essential to life processes (e.g., Eu$^{3+}$ replacing many Ca$^{2+}$ functions) in most species (Franklin, 2001). Now that the Lns are relatively available for biological functions, it may be that as plants continue to evolve while exposed to increasingly REE-rich topsoil environments, they are adapting to use certain REEs for specific metabolic and physiological processes, hence the hyperaccumulation in *Carya*, and especially in tetraploid *Carya*. Observations that certain REEs can increase photosynthesis, chlorophyll content, and nitrogen metabolism (Cao et al., 2007; Weiping et al., 2003; Xue and Yang, 2005; Yin et al., 2009), replace Ca$^{2+}$ in stomatal regulation (Xue and Yang, 2005), affect abscisic acid (ABA)-induced gene expression (Dagenbe et al., 2000; Rock and Quatrano, 1996), replace Ca in photosystem-II (Hong et al., 2002a, 2002b; Wang et al., 2003), replace Mg in the porphrin ring of chlorophyll (Hong et al., 2002a; Wei et al., 2005; Weiping et al., 2003), increase activity of nitrate reductase (Song et al., 2003) and glutamine synthase and glutamate dehydrogenase (Song et al., 2003; Wan et al., 2004), and improve plant growth (Boyko et al., 2011) of certain species is evidence that REEs might be beginning to be incorporated into the biology of certain species. The relatively high tolerance of most species to REEs might also be evidence of active positive biological roles. There is also evidence REEs benefit certain crop species in agricultural settings (Tyler, 2004); hence, they are widely used in Chinese agriculture with anticipation of positive effects on crops (Pang et al., 2002; Xu et al., 2002). Conversely, in U.S. and other non-Asian agricultural systems, REEs receive little or no attention, especially within the context of the physiology or horticulture of woody polycarpic perennial angiosperm type crops, although they are relatively common in many agricultural soils [i.e., Y at 16–33 mg kg$^{-1}$, LREEs $\approx$ 76–448 mg kg$^{-1}$, and HREEs of $\approx$ 9–55 mg kg$^{-1}$ and are commonly found to be high in many manures added to soils (Tyler, 2004)].

REEs are routinely applied to agricultural soils as impurities in phosphate fertilizers, but plant access to REEs in the soil solution is minimal as a result of their rapid precipitation with phosphate (Diatloff et al., 1993; Laveuf and Cornu, 2009). Thus, if biologically relevant, reduced bioavailability of REEs under high soil P or leaf P conditions and dry conditions might be expected to limit crop yield and/or quality. The selective accumulation of REEs, especially by tetraploids, identifies these taxa as a phenomenon of potential physiological and ecological uniqueness and raises questions regarding REEs relevance of to *Carya* nutritional physiology. The recent discovery that pecan possesses a relatively high Ni requirement during early spring (Wood et al., 2004), and Ni's associated importance in nitrogen, organic acid, and fatty acid metabolism.
and thrives; hence, any putative metabolic advantage during portions of the growing season than habitats where in habitats that are much drier, on upper terraces and low ridges, based on morphology group grouping pattern is also interesting in that taxa classification both species' ability to adapt to drier environments. This conferred by one or more REEs might well be operating in increased exposure to radioactive REEs might present inordinate risks to REE hyper-trophic REEs into the environment. This increased exposure to malfunctioning nuclear reactors can introduce certain radioac-
tive elements become either beneficial or essential (Mattson, 2008). Examples of positive hormetic outcomes include protection of cells or organisms against environmental stresses (e.g., drought, high temperature, toxic metals, and oxidative inducers), thus benefiting cell or organism health and survival fitness. Over evolutionary time, organisms can incorpo-rate hormetic agents into cellular processes to the point that these elements become either beneficial or essential (Mattson, 2008).

It is noteworthy that nuclear detonations and certain types of malfunctioning nuclear reactors can introduce certain radioactive REEs into the environment. This increased exposure to radioactive REEs might present inordinate risks to REE hyper-ac-cumulating Carya species such as pecan and therefore adversely affect subsequent seed germination and seedling survival as well as the health of animals and humans consuming the nutmeats (Thomas, 1975).

**Potential ecological role of rare-earth elements in Carya species and speciation.** If substitution by REEs occurs in certain metalloenzymes or proteins, then there is potential for existence of enzyme isoforms that benefit species adaptation. REE accumulation might confer a degree of metabolic and ecological flexibility absent in many non-Carya species; thus, certain REEs might function as beneficial, or even essential, nutrient elements for trees exposed to certain environmental stresses. This could theoretically be a factor contributing to speciation of tetraploid Carya and their successful colonization of specific habitats not well occupied by diploid Carya. Such is not only relevant to the evolutionary biology and ecophysiology of Carya, but also the horticulture of pecan and other Carya.

Based on distance measures of hierarchal cluster analysis, diploid species possessing the most similar REE metallomes are C. illinoinensis and C. myristiciformis. Both species can grow in habitats that are much drier, on upper terraces and low ridges, during portions of the growing season than habitats where C. aquatica thrives; hence, any putative metabolic advantage conferred by one or more REEs might well be operating in both species' ability to adapt to drier environments. This grouping pattern is also interesting in that taxa classification based on morphology group C. myristiciformis with section Carya and C. illinoinensis and C. aquatica with section Apocarya.

Although C. myristiciformis substantially differs from the other two morphologically, it retains substantial similarity in its REE metallome. The similarity between C. ovata and C. septemtrio-nalis is also not surprising in that both are morphologically similar and considered by some to be biotypes of the same species; however, possession of distinct quantitative different in their REE metallomes indicates that they are at least distinct biotypes and may well be different species after all.

In the case of tetraploid taxa, the REE metallomes of C. tomentosa and C. glabra are distinctly different from that of the other three tetraploids with both species occupying seasonally xeric ridges and hills within their natural habitats. Similarly, C. floridana and C. pallida also occupy similar seasonally xeric habitats, but their native ranges do not overlap. The native range of C. texana does not overlap that of either C. pallida or C. floridana and possesses greater dissimilarities in REE metallome composition. The especially high accumulation of REEs by tetraploid species hint that these elements might play important fitness-associated biological roles. Although REE accumulation may simply be an artifact without selective advantage, it is also possible that one or more of the REEs are “beneficial” to Carya species or may even be “essential.”

Several primitive plant taxa accumulate REEs, regardless of soil REE content, compared with more evolutionarily advanced cycadales, conifers, and angiosperms (monocots and dicots). Examples include many ferns (Polypodiophytina), horsetails (Equisetophytina), and clubmosses (Lycopodiophytina) (Ozaki et al., 2000; Watanabe et al., 2007). Wytenbach et al. (1996, 1998) noted that REE concentration in most plants is in the parts per billion range with certain fern species accumulating La and Nd as high as ≈635 μg g⁻¹ dry weight. The present study not only confirms previous reports that C. tomentosa accumulates REEs (Robinson, 1943; Robinson and Edgington, 1945; Robinson et al., 1938, 1958; Thomas, 1975), but that almost all indigenous U.S. species are significant REE accumulators.

Topsoils, especially the humus zone, are generally enriched with REEs through plant recycling with organic matter possessing an especially high capacity to complex, adsorb, or chelate positively charged REEs. The organic matter in topsoils is usually enriched more, in a relative sense, with HREEs and MREEs than LREEs (Laveuf and Cornu, 2009); thus, soils high in organic matter such as those in which most wild Carya species grow in their native habitats potentially have positive HREE and MREE anomalies (Thomas, 1975). Decomposing leaf litter can lead to a center of REE concentration that tends to perpetuate itself, exemplifying the role of Carya taxa as accumulators influencing distribution of elements within their ecosystems. The hickories are centers of REE concentration and therefore reduce losses from the tree-soil system where they are major components. Under soil conditions with relatively high REE availability, it is possible that over evolutionary time Carya species have in-tegrated certain REEs into life processes to such a degree that they are at least beneficial, if not essential. This merits further study, especially within the context of pecan horticulture.

It is noteworthy that the natural habitats of all tetraploid North American Carya are substantially more xeric during portions of the growing season than the relatively mesic habitats of diploid Carya. This suggests that REEs might confer a survival advantage linked to water relations. Nutrient availability from dry soils is minimal; thus, REEs might benefit specific xeric-associated metabolic processes when other nutrients are insufficiently available or, perhaps more likely, benefit water relations and
drought tolerance. Additional evidence for a REE role under xeric conditions is their apparent ability to replace Ca\(^{2+}\) in stomatal regulation (Xue and Yang, 2005) and elicitor activity for ABA-associated gene expression (Rock and Quatrano, 1996).

Although plant reliance on a particular element requires substantial evolutionary time, there has nevertheless been sufficient time to enable enough evolutionary diversification for integration of one or more REEs into the metabolome of at least a few modern species (Wytenbach et al., 1998). REE accumulator species such as tetraploid Carya might well be the vanguard taxa for such a role.

**Summary**

The present study 1) provides first-time qualitative and quantitative characterization of the REEs metabolome as a component of Carya foliar ionomes and a comparison of the metabolomes of 12 North American Carya species; 2) identifies most Carya species as REE accumulators; 3) establishes linkage between ploidy level and REE accumulation with tetraploid Carya being hyperaccumulators; 4) identifies among species relationships based on the REEs metabolome; 5) identifies a similar, relatively non-discriminatory, accumulation process for the various REEs; 6) identifies relationships between accumulation of specific REEs and essential macro- and micronutrients; 7) provides limited circumstantial evidence for a role for REEs in the Carya metabolome; 8) identifies the hickories as being at risk of accumulation of radioactive REEs emanating from nuclear explosions or malfunctioning nuclear reactors; and 9) provides evidence that REEs confer an adaptive advantage to tetraploid taxa for adapting to relatively xeric habitats with improved drought tolerance being at least one benefit. REEs are likely important contributors to Carya ecophysiology and therefore potentially play important roles in the nutritional physiology of horticulturally important Carya such as pecan. Thus, REE-associated nutritional physiology of tree organs might influence key physiological processes such as tolerance to certain abiotic and biotic stresses. We postulated that certain REEs play important roles in Carya as pertaining to environmental signaling; thus, REE nutritional physiology merits further study to ascertain their metabolic, ecophysiological, or horticultural relevance. It is noteworthy that elemental essentiality has been determined for relatively few plant species with even fewer being woody perennials. Considering the enormous diversity of the plant kingdom, it is possible that one or more REEs are either beneficial or essential to certain species. Their hyperaccumulation in tetraploid Carya taxa, and the adaptation of these taxa to relatively xeric sites, is suggestive that the biological Periodic Table for pecan, and most other North American hickories, might include one or more of the REEs as beneficial or essential nutrient elements.

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