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Influence of lanthanum level and interactions with nitrogen source on early development of *Juglans nigra*

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Abstract: Rare earth elements have been used for 30 years in Chinese agriculture to improve growth and yield. Numerous scientific studies have shown improvements in physiology, mineral nutrition, and growth, though deleterious effects may also occur. Very few studies have been performed on woody species. We exponentially fertilized one-year old black walnut (*Juglans nigra* L.) seedlings with 0, 0.1, 1, 10, or 100 mg lanthanum (La) and 800 mg nitrogen (N) as $NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3, or no N. One month following final fertilization, growth, mineral nutrition, photosynthesis, chlorophyll, and nitrate reductase activity were assessed. Plants fertilized with the highest level of La had reduced fine root growth, concentrations of magnesium, calcium, nickel, and phosphorus, photosynthesis levels, and chlorophyll *a* content. Foliar La concentration showed an interaction effect, with three to four times greater concentration in plants fed at 100 mg La to those given 10 mg La for $(NH_4)_2SO_4$ and NH_4NO_3 treatments. The results suggested no beneficial effects of La addition at levels used in this study and interactions between N source and La levels did not have an important impact on the growth, mineral nutrition, or physiology of black walnut seedlings.

Keywords: lanthanum; nitrate reductase; chlorophyll; photosynthesis; *Juglans nigra*; rare earths

Rare earth elements (REEs) are metallic ions comprising elements in the lanthanide series from lanthanum (La) to lutetium (Lu), and commonly also include scandium (Sc) and yttrium (Y)[1]. The REEs are stable except promethium (Pm), which is radioactive. The REEs are not always "rare" in terms of availability. Cerium (Ce) is the 25th most abundant of the 78 common elements in the Earth's crust, and the second least abundant REE, Lu, is 61st most abundant[1].

The amount of agronomic land in China fertilized with REEs may range from 3.0×10^6 to 2.7×10^8 ha y^{-1}[2,3], which may be associated with increases in dry mass of plants in the range of 8%–25% (reviewed in[4–6]). Positive effects of REEs follow a typical nutrient dose response curve, with induced toxicity at higher levels[7] (reviewed in[5]). Decreases in dry mass, however, have also been found[8,9].

The effects of REEs on plant mineral nutrition are quite variable (reviewed in[4]). Studies have shown increased and decreased concentrations for every element examined. We would expect REEs to initially limit uptake of other cations or release cations into the rhizosphere as charge compensation. Anions, acting as counterions, have been shown to increase REE uptake[10]. After REEs are transported from the region of uptake, cations could begin to be absorbed. With foliar spray of neodymium (Nd), nitrogen (N), phosphorus (P), and potassium (K) increased in sprayed leaves but decreased in non-sprayed leaves, suggesting that REE application may affect element translocation[11].

Most of the studies in the literature that quantified calcium (Ca) concentration showed decreases with REE application[9,11]. Lanthanum has been used as a Ca antagonist[12]. The ionic radii of REEs are similar to Ca[5], so REEs and Ca could compete for sites in the plant that normally bind Ca[13]. Additionally, REEs could cause limitations in uptake of other ions by mechanisms similar to those of Ca[14]. Calcium could also be limited by REE-induced changes in physiology or REEs replacing Ca[15,16].

Many studies have been conducted on physiological effects of REE application (reviewed in[5,6,17]). Rare earth elements have increased photosynthesis and chlorophyll content and promoted N metabolism. Lanthanum can replace Ca in functions such as regulating stomatal movement[16] and affecting ABA-inducible gene expression[18]. Rare earth elements can also prevent the activity of Ca in dielectropho-

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resis[19] and gravitropism[12]. The varied response in relation to Ca probably relates to decreases in Ca uptake balanced by replacement of Ca by REEs. Studies have shown that REEs can replace Ca in binding to photosystem II (PS II) with higher activity[20,21], increasing the rate of photosynthesis. The Mg in the porphyrin ring of the chlorophyll molecule can also be replaced by REEs[22,23]. Nitrate reductase activity (NRA) was increased[24] and both glutamine synthetase and glutamate dehydrogenase were increased with REE addition[24,25].

Most of the studies in the literature have been conducted on agronomic crop species (Tables 1, 2). The two studies just mentioned on N metabolism were conducted on loquat (*Eriobotrya japonica* (Thunb.) Lindl.) and peach (*Prunus persica* (L.) Batsch)[24,25], which are woody species. These studies were conducted on rooted cuttings in tissue culture, averaging in size < 2 cm, so it is questionable how well these results would apply to larger plants. It is very important to find out how REEs impact the growth and physiology of woody species, to know if the tremendous benefits found in agronomic species may be transferred to forestry.

We are aware of no published studies that quantified interaction effects between N fertility and REEs, though N fertility is known to influence many of the processes that are affected by REEs. Due to charge compensation, we would expect uptake of nitrate (NO_3^-), to be increased with the addition of REEs and for NO_3^- to promote REE uptake[10]. Uptake of ammonium (NH_4^+) is likely to be depressed with REE element uptake. Further, we would anticipate REE membrane binding[7] to impact uptake of NH_4^+, since NH_4^+-uptake requires a change in membrane polarization.

Some studies suggest that La may stimulate root growth and development as well as significantly enhance NRA[26,27]. In this study, we examined the effects of La level and N source on C and N metabolism, growth, and mineral nutrition of black walnut (*Juglans nigra* L.) seedlings. We expected (1) La addition to decrease Ca concentration and have significant and varied effects on other mineral nutrient elements, (2) La to show favorable increases in growth, chlorophyll content, photosynthesis, and NRA at low levels, followed by decreases as La levels reach toxicity, and (3) positive effects of La addition to be increased with NO_3^- more than with no N, NH_4^+, or a mixed N source.

1 Methods

1.1 Plant material and treatments

Half-sib (from expired patent Purdue #1 mother tree) black walnut seedlings were grown under operational bare-root production systems for one season in the Indiana State Nursery at Vallonia (38°85'N, 86°10'W). Seedling production protocols are detailed in Jacobs (2003). In October 2005, seedlings were lifted and overwintered (3 °C to 5 °C) in a cooler at the Purdue University Horticulture Farm. In April 2006, seedlings were removed from cold storage and potted into 10.65 l Treepot™ containers (Steuwe and Sons, Corvallis, OR, USA) at the Purdue University Horticultural Plant Growth Facility (40°4'N, 86°30'W). The planting medium, Berger BM-6™ (Berger Peat Moss, St. Modeste, Quebec, Canada) consists of 4:1 peat:perlite. Plants were watered equally twice a week as needed (~1l $plant^{-1}$ $week^{-1}$) based on gravimetric techniques, with pH adjusted to ~6.0, which is known to be within the ideal range for growing walnut seedlings (Williams 1990). Greenhouse air temperature ranged from 20 °C to 24°C and supplemental light (350 μmol/m^2/s to 400 μmol/m^2/s) was used to provide 16 h of daylight.

Half-sib (from expired patent Purdue #1 mother tree) black walnut seedlings were grown and treated as in our previous studies[29,30]. Plants were fertilized with N as NH_4NO_3, $NaNO_3$, or $(NH_4)_2SO_4$ at 800 mg N $plant^{-1}$ $season^{-1}$ or 0 mg N and with La at 0, 0.1, 1, 10, or 100 mg La $plant^{-1}$ $season^{-1}$. The N rate showed a significant positive growth responses in previous work with black walnut[28,29]. As much as 766.7 mg La $plant^{-1}$ has been used in studies with crop species, but positive results have seldom occurred at a rate greater than 25 mg La $plant^{-1}$[30,31], and the rates used in Chinese agriculture are generally much lower[4–6]. We selected a range covering 4 orders of magnitude to determine both the levels that have positive effects and the levels that show toxicity. The experimental design was a 4×5 (N source *x* La rate) factorial with 7 replications. Initial N content was determined from 5 plants, which was used to compute exponential fertilization regimes as detailed elsewhere[32]. The total seasonal fertilizer dose (N_T) was supplied over 7 applications. Exponential fertilization was used to help maximize plant nutrient uptake and physiological response to experimental treatments. Exponential fertilization has been successfully applied to one-year-old conifer seedlings[33] and for seedling culture of other hardwood species[34,35]. Lanthanum was added along with acetic acid (3:1) as a chelator[3], and pH of the fertilizer solution adjusted to ~4[36]. Since only 5 ml of solution was added at La fertilization, and plants were receiving ~11 of water a week, the adjusted pH of the solution did not have a large effect on the total pH of the soil solution. One month following the final fertilizer application, physiological measurements were conducted and seedlings were processed to determine dry weight of components and nutrient concentration. Component N content was computed as concentration multiplied by

plant dry mass.

1.2 Physiological measurements

Nitrate reductase activity was assessed using an *in vivo* assay as detailed in[28]. Photosynthesis was measured with an LI-6400 portable photosynthesis system (Li- Cor Inc., Lincoln, NE, USA) and chlorophyll was assessed by colorimetric assay as described in[29].

1.3 Plant sampling, chemical, and statistical analysis

Plants were separated into upper and lower leaves, fine and coarse roots, new and old stems following physiological measurements. New stems were those taken from the stem between the location of the initial terminus (at planting) and the final terminus (at harvest). Fine roots were those <2 mm in diameter. Mineral nutrients and La were measured by ICP-MS on 5 mg fresh mass leaf samples after dissolution in nitric acid[37]. Following ICP-MS analysis, plant components were placed in an oven for at least 72 h at 70 °C and component dry mass determined. Subsequently, samples were ground with a Wiley mill (Thomas Scientific, Swedesboro, NJ, USA) to pass a 20 mesh sieve. For carbon (C) and N determination, 200 mg of sample was used. Carbon and N were determined by combustion using a LECO CNS-2000 analyzer (Leco Corporation, St. Joseph, MI, USA).

Analysis of variance (ANOVA) was conducted on measured data with treatment effects tested at $P<0.05$ and where significant, treatment means were ranked according to Tukey's honestly significant difference (HSD) test at $\alpha=0.05$ using SAS software (SAS Institute, Cary, NC, USA). To minimize the possibility of making a Type II error for some N source and interaction effects, ANOVA treatment effects were considered significant for these variables at $P<0.10$ and where significant, treatment means were ranked according to Tukey's HSD test at $\alpha=0.10$. Prior to ANOVA, data were tested and found to meet the ANOVA assumptions for normality (Shapiro-Wilks test) and homogeneity of variances (Bartlett test). The linear model for the ANOVA is given as:

$$Y_{ijk} = m + \mathrm{La}_i + \mathrm{N}_j + \mathrm{LaN}_{ij} + e_{(ij)k}$$

where, Y_{ijk} is plant component dry mass, or measured physiological response of the kth replicate (k=1, 2, 3,…, 7), estimated from the jth N source (j=1, 2, 3, 4) from the ith La level (i=1, 2, 3, 4, 5); m=overall mean; La_i=fixed effect of the ith La level; N_j=fixed effect of the jth N source; followed by the interaction effects and ε is error associated with measured seedling dry mass or physiological response from bulk replicates.

2 Results

2.1 Growth

Total plant dry mass was not significantly affected by N source or by La level (Table 1). The NO_3^--treated seedlings did have 18.9% greater dry mass than seedlings given no N, with the other treatments intermediate (Fig.1(a)), but this was not significant. The seedlings decreased in dry mass with La addition (Fig.1(b)), with plants given no La 13.2% larger than seedlings treated with 100 mg La plant^{-1}.

Fine root dry mass was significantly affected by La level (Table 1). Plants fertilized with 1 mg La plant^{-1} and 0 mg La plant^{-1} had 73.8% and 70.2% greater fine root dry mass than those fertilized at 100 mg La plant^{-1} with the 0.1 and 10 mg La plant^{-1} seedlings being intermediate (Fig.1(b)). Old stem dry mass was affected by N source (Table 1), with the NO_3^--treated seedlings having 20.8% greater old stem dry mass than unfertilized N seedlings (Fig.1(a)).

Table 1 ANOVA of parameters measured in this study*

	Dry mass						Total nitrogen					
Sources of variation	Fine root	Coarse root	Old stem	New stem	Leaves	Plant	Fine root	Coarse root	Old stem	New stem	Leaves	Plant
N source	0.6881	0.2601	***0.0862***	0.8678	0.1942	0.1633	0.1258	**0.0278**	**0.0369**	***0.0925***	**0.0015**	**0.0008**
La level	**0.0174**	0.5990	0.9554	0.6444	0.3298	0.4620	**0.0460**	0.7267	0.8585	0.8521	0.2577	0.4671
Interaction	0.6109	0.8533	0.1319	0.3375	0.8878	0.7218	0.5606	0.7856	0.9022	0.2276	0.9150	0.7080
Sources of	Chlorophyll: *a*		*b*		Total	Photosynthesis			NRA			
variqation	Upper	Lower	Upper	Lower		Upper	Lower	Total	Upper	Lower	Roots	
N source	**0.0013**	0.2026	0.1617	***0.0601***	**0.0141**	0.2960	**0.0049**	0.7781	**<0.0001**	***0.0994***	**0.0068**	
La level	**0.0049**	0.2446	0.4681	0.6789	0.2943	***0.0901***	0.1086	0.1583	0.6374	0.6792	0.2201	
Interaction	**0.0030**	0.5350	0.7754	0.7795	0.6017	0.5842	0.1101	0.7740	0.5549	0.8893	0.8967	

* Bold *P* values indicate significant differences at $\alpha=0.05$. Bold italicized *P* values indicate significant differences at $\alpha=0.10$

2.2 Mineral nutrition

The concentration of La in the leaves of black walnut seedlings was significantly affected by the interaction of N source with La level (Table 2). With NH_4^+ or NH_4NO_3 as the N source, there was a significant increase in leaf La concentration (Fig.2). The La concentration was 3.49 times and 4.24 times greater at the 100 mg La plant^{-1} than the 0.1 mg La plant^{-1} level for seedlings fertilized with NH_4^+ and NH_4NO_3, respectively, and the disparity between the 100 mg La plant^{-1} level and the other treatment levels was even greater. There was no significant difference in La concentration in plants given no N or NO_3^- (Fig.2).

Mg, P, Ca, and Ni concentrations were all significantly affected by La level (Table 2). Magnesium concentration was significantly greater in the 0 and 10 mg La plant^{-1} treatments than the 100 La plant^{-1} treatment, with the 10 mg La plants having a 20.4% greater Mg concentration than the 100 mg La plants. Plants fed with 10 mg La had 34.8% greater P concentration than those given 100 mg La. Calcium was greater with 0, 1, or 10 mg La than 100 mg, with Ca concentration 25.0% greater in the plants fed with 10 mg La than those fed with 100 mg La. Nickel concentration was greater in plants given no La or 1 mg La than those given 100 mg La. Calcium and boron (B) were also significantly affected by N source, with the concentration of Ca being 17.8% with NH_4^+ than no N and B concentration 27.9% greater with NH_4^+ than with NO_3^- (Table 2).

Nitrogen concentration (Table 3) and content (Fig.1(c)) for most components and the entire plant were significantly affected by N source, with one or more N types being significantly greater than the no N treatment. This was reflected in the greater C:N for all components for the no N treatment than those given N (Table 3). Nitrogen concentration was higher in fine roots for plants given 0, 0.1, and 100 mg La than those given 1 mg and in coarse roots for those given 10 and 100 mg La than those given 1 mg La (Table 3). Fine root N content was 70.3% greater for plants given 0 mg La than those given 100 mg La. The C:N for fine roots, coarse roots, and upper leaves were all affected by La level (Table 3). Plants given 1 mg La had higher C:N for coarse and fine roots than for plants given more or less La. Upper leaves had higher C:N for plants given 100 mg La than those given less La.

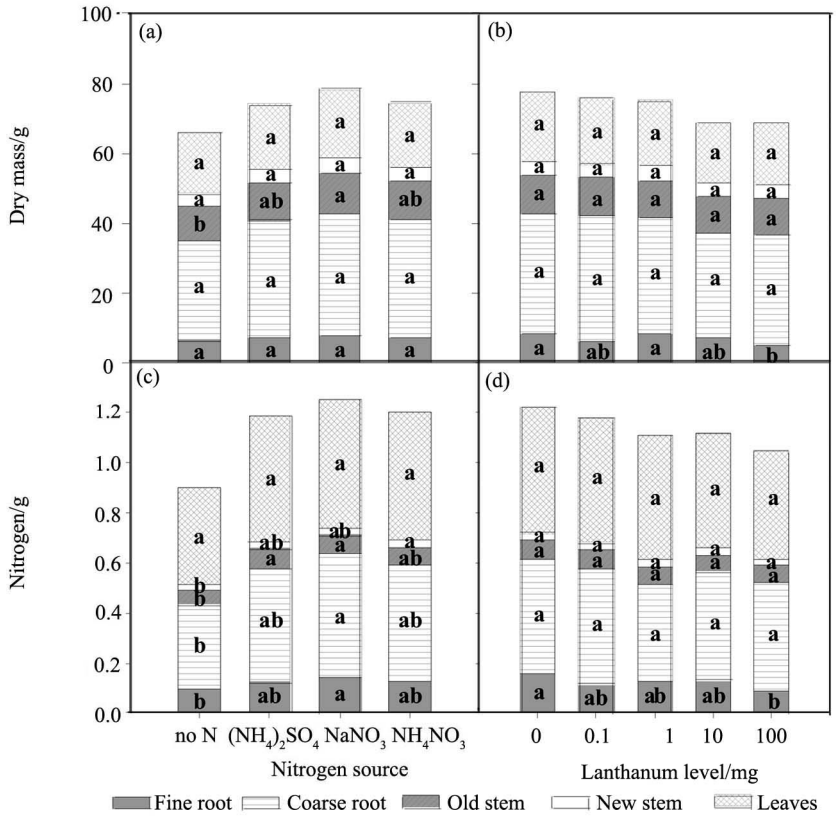

Fig.1 Black walnut seedling dry mass (g) by component as affected by N source (a) or La level (b), and N content (g) as affected by N source (c) or La level (d) (Lowercase letters show significant differences (Tukey, α=0.10) between treatments within each component)

Table 2 Essential nutrients, sodium, and lanthanum in black walnut seedlings as affected by nitrogen source or lanthanum level*

Parameters	Elements (g/kg)					Elements (mg/kg)							
	Mg	P	K	Ca	Mn	Fe	Ni	Cu	Zn	Mo	B	Na	La
N source													
No N	16.7a	9.21a	37.7a	42.6b	4.91a	321a	13.0a	10.7a	470a	1.16a	153ab	346a	0.252a
A	17.7a	9.20a	32.5a	50.2a	5.21a	390a	15.0a	9.98a	378a	1.08a	165a	344a	0.397a
N	16.5a	7.78a	32.6a	45.0ab	5.91a	291a	13.0a	9.14a	359a	0.950a	129b	398a	0.257a
AN	16.4a	8.07a	33.1a	46.1ab	4.61a	367a	13.8a	9.25a	313a	1.00a	147ab	275a	0.382a
P value	0.3323	0.2927	0.1306	**0.0128**	0.7898	0.2247	0.1389	0.6550	0.4955	0.3696	***0.0583***	0.5352	0.2307
La level (mg)													
0	17.2a	8.66a	31.8a	48.8a	4.67a	290a	14.7a	10.3a	427a	1.05a	143a	357a	0.253b
0.1	16.8ab	9.49a	35.0a	46.1ab	5.96a	313a	13.7ab	8.81a	399a	0.952a	152a	250a	0.283b
1	16.7ab	7.74a	34.6a	46.1ab	5.27a	364a	14.1a	9.12a	442a	1.15a	152a	452a	0.232b
10	18.3a	9.73a	35.2a	49.5a	5.42a	380a	14.3a	10.1a	313a	1.14a	167a	294a	0.205b
100	15.2b	7.22a	33.5a	39.6b	4.59a	368a	11.6b	10.5a	317a	0.942a	127a	347a	0.628a
P value	**0.0114**	***0.0753***	0.7985	**0.0038**	0.9299	0.4517	**0.0258**	0.6206	0.5984	0.2685	0.1531	0.2366	**<0.0001**
Interaction *p* value	0.9445	0.8470	0.4073	0.8416	0.4664	0.8396	0.8008	0.9317	0.9741	0.5731	0.9906	0.8632	**0.0409**

* Bold *P* values indicate significant differences at α=0.05. Bold italicized *P* values indicate significant differences at α=0.10. Column means within treatment labeled with different letters differ significantly according to Tukey's HSD test at α=0.05

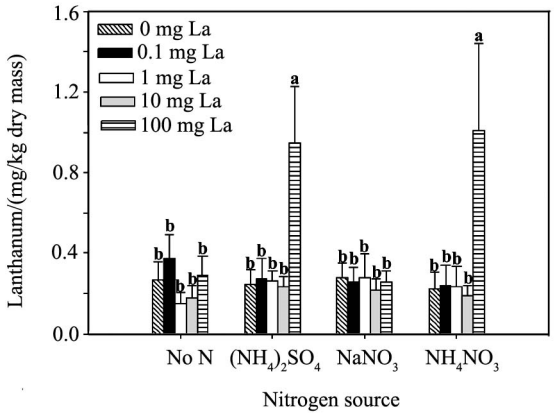

Fig.2 Black walnut leaf La concentration (mg/kg dry mass), N source by La level interaction (Lowercase letters show significant differences (Tukey, α=0.10) between treatments)

2.3 Physiology

Photosynthesis was significantly lower (Table 1) in lower leaves with NO_3^- as the N source than with other sources or with no N, with the mixed source having a 26.7% greater photosynthetic rates than NO_3^- fertilized plants (Fig.3(a)). Photosynthesis in upper leaves was significantly lower in plants given 100 mg La (Table 1) than in any other La treatments, with 0 mg La treated plants having 21.3% greater photosynthetic rates (Fig.3(b)). Photosynthesis was consistently higher in upper than in lower leaves (Fig.3(a), (b)).

Chlorophyll *a* in the upper leaves was significantly affected by N source and by La level. Plants fertilized with N had higher chlorophyll *a* (Table 1) than those given no N

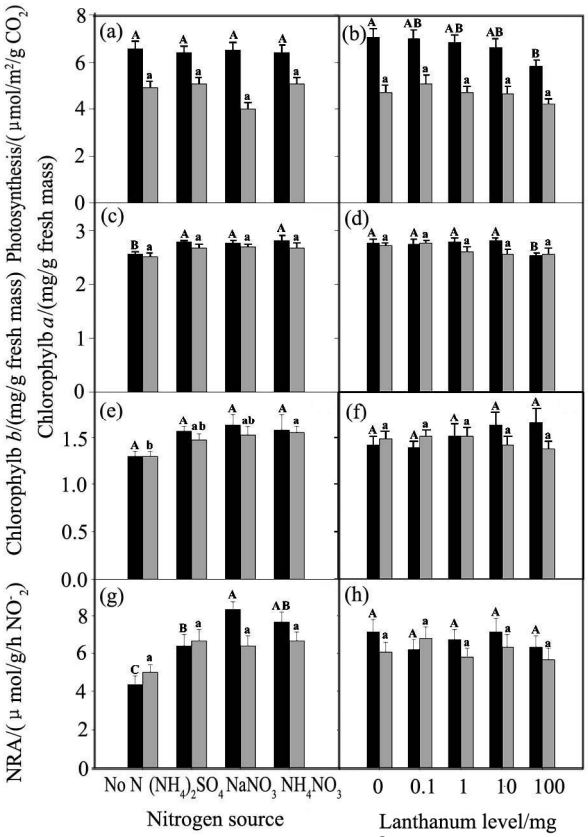

Fig.3 Black walnut photosynthesis (μmol/m^2/s CO_2) as affected by N source (a) or La level (b), chlorophyll *a* content (mg/g fresh mass) as affected by N source (c) or La level (d), chlorophyll *b* content (mg g^{-1} fresh mass) as affected by N source (e) or La level (f), and NRA (μmol NO_2^- g^{-1} h^{-1}) as affected by N source (g) or La level (h) (Uppercase letters show significant differences between treatments (Tukey, α=0.10) in upper leaves (black bars) and lowercase letters show significant differences between treatments (Tukey, α=0.10) in lower leaves (gray bars))

(Fig.3(c)). Plants given 100 mg La had lower chlorophyll *a* than plants given less La, with the plants given 10 mg La having 11.65% more chlorophyll *a* (Fig.3(d)). There was also significantly less chlorophyll *b* in lower leaves (Table 1) and the mixed source had 19.1% more chlorophyll *b* than the plants given no N (Fig.3(e)). Chlorophyll *a* was generally greater in upper leaves than in lower leaves.

Nitrate reductase activity was significantly greater in upper (P<0.0001) and lower leaves (P=0.0994) as well as in roots (P=0.0068) with NO_3^- than with no N (Table 1). In the upper leaves, there were three levels of separation of means by Tukey, with the plants given NO_3^- having greater NRA than those given NH_4^+, and those given NH_4^+ had greater NRA than those given no N (Fig.3(g)). La levels showed no significant trend in NRA (Fig.3(h)).

3 Discussion

3.1 Growth

In reviews of the literature, gains in dry mass with REE addition ranged from 8%–25%[5,6]. In this study, there were no beneficial effects of adding La to black walnut seedlings in terms of plant biomass, in fact there was a steady decline in dry mass with La addition, though insignificant. This corresponds with other studies that have shown a decrease in biomass with REE addition[8,9]. The decreased root dry mass with increasing La observed in this study (Fig.1(b)), contrast markedly with improved root growth with La noted in other studies[26,27]. The decreased root dry mass with La concurs with results noted by[7], which may be associated with a toxic response at higher La levels. Plants responded with a predictable, though insignificant, increase in biomass with N addition compared to no N. There were no significant interactions between La level and N source, suggesting that there was no strong effect on plant biomass with La addition with different N sources.

3.2 Mineral nutrition

La concentration in the leaves of black walnut seedlings was only significantly increased at the 100 mg La plant^{-1} level (Table 2). This increase was only significant in plants fertilized with $(NH_4)_2SO_4$ and NH_4NO_3 (Fig.2). We expected that the uptake of La would be increased with $NaNO_3$, since NO_3^- would act as a counterion for La^{3+} to balance the charge in the roots following ion uptake. Lanthanum is often applied as $La(NO_3)_3$[38,39], so it would stand to reason that there would be a charge balance effect in La uptake with NO_3^-. A possible mechanism for the opposite effect is that La can be strongly adsorbed to soils depending on soil type[6]. This would likely be due to the cationic exchange capacity (CEC) of the soil. Since NO_3^- uptake is balanced by the uptake of a H^+, the pH of the soil would increase, which has an adverse effect on La uptake[36], partially because of a decrease in the amount of La^{3+} bound to soil components with a high CEC. It is possible that La was taken up by the roots at levels other than the highest rate, but the ions were unable to be transferred to the shoot due to restrictions on apoplastic transport of La into the xylem by the Casparian strip[40]. Alternatively, it could be that La was sequestered in the stem as has occurred in some other hardwood species[41].

The concentration of La in seedlings given 100 mg La and NH_4^+ (0.947 mg/kg dry mass) or NH_4NO_3 (1.01 mg/kg dry mass) in this study were generally mid-range compared to unfertilized concentrations of La found in other studies on hardwood species. Beech (*Fagus sylvatica* L.)[42] leaves had 0.044–0.049 mg/kg La dry mass and mulberry (*Morus bombycis* Koidz.)[43] leaves had 0.46 mg/kg La dry mass, which are lower than our results. Aspen (*Populus sieboldii* Miq.) had 1.17 mg/kg La dry mass[44], which is very similar to the results we found. In the NIST SRM 1515 apple leaves standard and in the NIST SRM 1547 peach leaves, the concentration of La was 20.4 and 8.9 mg/kg La dry mass respectively[45].

Calcium and Mg concentrations were significantly lower for seedlings fed with 100 mg La than those given 0 or 10 mg La (Table 2). We expected the decrease in Ca concentration, though previous studies have reported mixed results for Mg concentrations (reviewed in[4]). Decreased Ca and Mg concentrations could relate directly to the significantly lower fine root dry mass in the seedlings given 100 mg La than the dry mass in the other La treatments, which would significantly decrease the surface area of roots available for nutrient uptake. It is also possible that some Ca^{2+} and Mg^{2+} ions could have acted as counterions for La^{3+} uptake, though this would not explain the levels of decrease seen in this study (Table 2) unless much of the La that was taken up remained in the root and was not transported in the xylem[40].

Nitrogen concentration was predictably increased with N addition in most of the plant components. Nitrogen concentration in fine and coarse roots was lower for plants fed 1 mg La than those given more or less La and there was a similar trend in S concentration on fine roots (Table 3). The lower N concentration may suggest that there was a significant effect on translocation of N in the plant with 1 mg of N. In both root components, the lower N concentration did not translate to a lower N content for the 1 mg La treatment. In fine roots, N content was much lower with 100 mg La than with other levels of La (Fig.1(d)). This would mostly be due to much lower fine root dry mass with 100 mg La. Total N content

Table 3 Sulfur and nitrogen percentage, and C:N for various components of black walnut seedlings as affected by nitrogen source or lanthanum level*

Treatment	Measured parameter	Plant component					
		Fine root	Coarse root	Old stem	New stem	Lower leaf	Upper leaf
Nitrogen source							
	S (g/kg)						
No N		2.29b	1.52a	1.14a	1.34a	2.19a	2.53b
$(NH_4)_2SO_4$		2.73a	1.62a	1.21a	1.57a	2.36a	2.77a
$NaNO_3$		2.23b	1.67a	1.17a	1.97a	2.17a	2.61ab
NH_4NO_3		2.28b	1.63a	1.23a	1.39a	2.36a	2.55b
	P value	**<0.0001**	0.5025	0.8514	0.3896	0.1230	**0.0226**
	N (g/kg)						
No N		15.65b	11.82b	6.21a	6.65b	21.13b	24.57b
$(NH_4)_2SO_4$		17.92a	13.42ab	7.58a	8.16a	24.52a	29.76a
$NaNO_3$		18.33a	14.20a	6.72a	7.53ab	23.74a	28.82a
NH_4NO_3		18.10a	13.56ab	7.12a	7.87a	24.53a	30.07a
	P value	**0.0116**	***0.0703***	0.5478	**0.0080**	**0.0001**	**0.0001**
	C:N						
No N		32.624a	52.453a	108.91a	81.393a	23.395a	20.616a
$(NH_4)_2SO_4$		26.812b	35.955b	79.63b	62.334b	19.936b	16.679b
$NaNO_3$		26.247b	35.118b	87.97ab	68.050b	20.630b	17.376b
NH_4NO_3		26.339b	36.595b	82.68ab	63.542b	19.832b	16.832b
	P value	**0.0010**	**0.0078**	***0.0517***	**0.0023**	**<0.0001**	**<0.0001**
Lanthanum level							
	S (g/kg)						
0		2.45a	1.52a	1.25a	1.34a	2.32a	2.66a
0.1		2.55a	1.73a	1.20a	1.48a	2.31a	2.64a
1		2.15b	1.55a	1.16a	1.44a	2.14a	2.53a
10		2.27ab	1.65a	1.15a	2.09a	2.25a	2.72a
100		2.50a	1.60a	1.17a	1.49a	2.18a	2.52a
	P value	**0.0017**	0.3541	0.9184	0.4722	0.2628	0.1626
	N (g/kg)						
0		18.22a	13.31ab	7.05a	7.24a	23.32a	28.00a
0.1		18.01a	13.44ab	6.92a	7.96a	24.24a	28.54a
1		15.40b	11.22b	6.77a	7.11a	22.60a	29.83a
10		17.61ab	14.27a	6.73a	8.01a	24.49a	29.12a
100		18.27a	14.02a	7.07a	7.44a	22.77a	26.03a
	P value	**0.0254**	**0.0329**	0.9970	0.2662	0.1626	0.1139
	C:N						
0		27.239b	39.542ab	89.15a	71.585a	20.970a	17.719ab
0.1		26.816b	36.306b	83.89a	64.775a	20.158a	17.416b
1		32.571a	52.744a	95.95a	74.115a	21.608a	17.633ab
10		26.919b	34.288b	90.68a	63.202a	19.942a	17.037b
100		26.482b	37.272ab	89.32a	70.472a	22.062a	19.573a
	P value	**0.0158**	**0.0395**	0.9234	0.3313	0.1070	**0.0340**

* Bold *P* values indicate significant differences at α=0.05. Bold italicized *P* values indicate significant differences at α=0.10. Column means within treatment labeled with different letters differ significantly according to Tukey's HSD test at α=0.05

was also lowest for plants given 100 mg La (not significant), which, compounded with the decrease in fine root dry mass, could be detrimental to plant growth in subsequent years as the plants would have less stored N and less fine root area for N uptake. Other than those mentioned for La concentration, there were no significant interaction effects for ion concentrations, so our assertion that charge balance would affect elemental uptake, particularly N, as La level increases based on N source were not verified.

3.3 Physiology

The decrease in photosynthesis in lower leaves (Fig.3(b)) and chlorophyll *a* in upper leaves (Fig.3(d)) in plants given 100 mg La compared to those given less La (Fig.3(b)) could be directly related to the decrease in Mg or Ca concentrations (Table 2) or the increase in C:N (Table 3). It was suggested that La can replace Mg in the porphyrin ring of chlorophyll and increase chlorophyll content[21,22]. Since the Mg concentration of the leaves was more than five orders of magnitude greater than the concentration of La (Table 2), any La-complexed chlorophyll would not make up for the decrease in Mg available for Mg-complexed chlorophyll. Lanthanum can replace Ca in PS II[22,23], but again the concentration of Ca was much greater than the concentration of La (Table 2), so any improvements to PS II with La would be counteracted by greatly decreased Ca available for PS II. The high C:N of the upper leaves with 100 mg La (Table 3) would also suggest that there would be less N available per unit C, which could decrease the content of N containing compounds such as RUBISCO and chlorophyll.

Nitrate reductase activity followed a predictable trend of increase with NO_3^- over NH_4^+ and with N addition as opposed to no N (Fig.3(g)). Nitrate reductase activity was not significantly affected by La level. This observation contrasts with improved NRA with REE addition noted in another study[24]. Iron is the metal in the porphyrin ring of nitrate reductase and iron level was not adversely affected by La addition, unlike Ca and Mg, so deleterious effects of toxic level of La would not be expected. There was also no apparent positive effect of La on NRA.

4 Conclusion

Very little interaction between N source and La level was observed in this study. The most important interaction was in terms of La concentration in the leaves, which was only significantly increased with NH_4^+ or a mixed N source but not with NO_3^- or no N. It could be that interactions of La with N source were more involved in translocation of La or that La uptake was adversely affected by NO_3^-, which was contrary to what we expected in terms of charge compensation. The lower concentration of N with 1 mg La addition in coarse and fine roots without a significant decrease in total N in the plant or in the organ lent further credence to the idea that there might be an effect on translocation. Most of the significant effects of La level were deleterious in nature and only occurred at the highest level, inferring toxicity. It was possible that La did not have the same positive effects on black walnut or other woody perennials as noted for other species investigated. It was also possible that positive effects might be possible with La addition but that positive effects would occur between 10 and 100 mg La. Future studies should more closely examine responses in range between these two values. Foliar application of La should also be considered, to ensure that more La would reach the leaves, without significant negative effect on uptake of other nutrients. Hydroponic growth and application of nutrients, which would likely increase the amount of La that would be available to the roots and reduce the amount that would be bound by the soil, represented another potential approach.

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