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The Potential of NO_3^--N Utilization by a Woody Shrub Species *Lindera triloba*:

A Cultivation Test to Estimate the Saturation Point of Soil NO_3^--N for Plants

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Responses of seedlings of a shrub species, *Lindera triloba*, grown in perlite culture medium, to nitrate (NO_3^--N) supply were investigated to estimate the saturating point of available NO_3^--N for plant utilization. NO_3^--N concentration and nitrate reductase activity (NRA) in leaves and roots were used as indicators of NO_3^--N uptake and assimilation by *L. triloba*. Root NRA increased with NO_3^--N supply when concentrations were low and reached a plateau at high NO_3^--N concentrations. On the other hand, root NO_3^--N concentration increased linearly with NO_3^--N supply; therefore, it is suggested that NO_3^--N uptake did not limit NO_3^--N assimilation by *L. triloba*. In contrast, leaf NRA and leaf NO_3^--N concentration were low and were not influenced by NO_3^--N supply. This may be caused by the lack of transport of NO_3^--N from roots to leaves. The NO_3^--N retained in perlite was compared with NO_3^--N pool sizes in soils from a forest where *L. triloba* occurs naturally to estimate the level of NO_3^--N availability to plants in the forest soil. The maximum NO_3^--N pool size in the forest soil was comparable to concentrations at which root NRA reached a plateau in perlite cultures. These results indicate that soil NO_3^--N availability is below the saturation point for NO_3^--N uptake by *L. triloba*, and it is the limiting factor of NO_3^--N utilization by *L. triloba* under field conditions in which this species naturally occurs.

KEY WORDS: nitrate reductase activity (NRA), nitrate (NO_3^--N) concentration, perlite, *Lindera triloba*

DOMAINS: plant sciences, enzymology, metabolism, nutrition, plant processes, physiology

INTRODUCTION

The increased nitrate (NO_3^--N) deposition derived from human activities has altered ecosystem nitrogen (N) cycles and has increased N availability to plants. It could, therefore, reduce the diversity in ecosystems over the long term[1]. Under changing regional or global N cycles, NO_3^--N uptake by plants is one of the most important processes in forest ecosystem N cycles. Because NO_3^--N is a highly leachable anion in forest soils, plant NO_3^--N uptake reduces not only N loss from ecosystem, but also other nutrient cations accompanied by NO_3^--N leaching[2]; therefore, work is being conducted to elucidate the importance of plant NO_3^--N use in N cycles in ecosystems[3,4,5], and information on the potential of plants for utilizing NO_3^--N is needed to assess the roles of plants influencing N retention by ecosystems.

Regarding assimilation processes of NO_3^--N by plants, the reduction of NO_3^--N to NH_4^+-N is required for the synthesis of organic N[6,7,8]. The first step after the uptake of NO_3^--N is the reduction of NO_3^--N to nitrite (NO_2^--N), and the process catalyzed by nitrate reductase (NR) is known to be the rate-limiting step in the sequence of NO_3^--N assimilation processes[7,9,10]; therefore, plant nitrate reductase activity (NRA) is a useful indicator of plant NO_3^--N utilization potential. Also, the existence of NO_3^--N in plant tissues can be evidence for plant NO_3^--N uptake, as plants do not synthesize NO_3^--N[11].

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The objectives of this study were to estimate the potential of NO_3^--N use by plants and to determine whether the NO_3^--N pool size in a forest soil exceeds plant NO_3^--N uptake potential. For these objectives, we selected a shrub species of Lauraceae, *Lindera triloba* (Sieb. et Zucc.) Blume. *L. triloba* was one of the dominant understory species in a conifer plantation (Koyama, unpublished data), where nitrification potential had wide range (0 to 12.2 mg N 100 g dry soil^{-1} 28 days^{-1})[12]. Experiments were conducted (1) to describe the responses of NO_3^--N use by *L. triloba* to NO_3^--N supply and (2) to examine the relationship of NO_3^--N supply to the amount of NO_3^--N retained in the cultivation medium and to compare this with the NO_3^--N pool size in forest soil. In seedlings of *L. triloba* grown in perlite medium supplied with various amounts of NO_3^--N, leaf and root NRA were measured, in addition to leaf and root NO_3^--N assays. The amount of NO_3^--N retained in perlite was compared with the soil NO_3^--N pool size in a forest where *L. triloba* is distributed.

METHODS

Plant Cultivation and Treatment

All seeds of *L. triloba* (Sieb. et Zucc.) Blume were collected from a single seed tree in Mt. Ryuoh in Shiga Prefecture, central Japan (35°10′N, 136°20′E) in September 1997. The collected seeds were stored at about 8°C until sowed in horticultural soil in April 1998. On April 29, 1999, seedlings were washed in tap water followed by deionized water to remove soil from roots. They were then individually transplanted into plastic pots filled with approximately 600 ml perlite that was prerinsed with deionized water. Throughout the period of the experiment, all seedlings were placed under a roof of a plastic film to keep out rain.

For 42 days after transplanting, each seedling was supplied daily with 200 ml nutrient solution containing 0.35 mmol l^{-1} $NaH_2PO_4 \cdot 2H_2O$; 0.63 mmol l^{-1} KCl; 0.5 mmol l^{-1} $CaCl_2 \cdot 2H_2O$; 0.25 mmol l^{-1} $MgSO_4 \cdot 7H_2O$; 59.37 µmol l^{-1} Fe-EDTA; 0.43 µmol l^{-1} Cu-EDTA; 0.42 µmol l^{-1} Zn-EDTA; 0.45 µmol l^{-1} Mn-EDTA; 32.35 µmol l^{-1} H_3BO_3; 0.41 µmol l^{-1} $Na_2MoO_4 \cdot 2H_2O$, and NO_3^--N. Nitrate was added in solution as $NaNO_3$ at 0, 1, 10, 25, and 50 ppm (molar concentrations were 0, 0.071, 0.71, 1.79, and 3.57 mmol N l^{-1}). Each of the five treatments was replicated ten times.

Plant Analysis

The leaves and roots of cultivated *L. triloba* were collected from 10:00 to 14:00 on June 8, 1999 at the 42nd day after the start of NO_3^--N additions. NRA was measured by a modified version of the *in vivo* test[13,14,15,16]. Samples were kept at 4°C until laboratory analysis. Two hundred leaf disks each with a diameter of 2.5 mm were cut out, and fine roots (diameter < 2 mm) were cut into about 5-mm lengths after being rinsed with deionized water. After vacuum infiltration (6 mm Hg; twice for 30 s each) with 5 ml of incubation buffer, the samples were incubated for 1 h at 30°C in the dark. The composition of the incubation buffer was 0.1 M KNO_3, 0.1 M KH_2PO_4, and 3% 1-propanol, and the pH was adjusted to about 7.5 with NaOH. Enzyme activity was stopped by placing sample vials in hot water (80°C). Leaves and roots were removed, oven-dried at 105°C, and then weighed to calculate the activity per unit dry weight. The concentration of NO_2^--N produced in the incubation buffer was measured colorimetrically by diazotization[17]. The effect of plant pigment was compensated for by measurement of complete controls lacking N-naphtylethylene diamine dihydrochloride.

The remaining leaves and fine roots were dried at 40°C and then ground. About 100 mg of ground sample was extracted with 10 ml of deionized water for 1 h at 45°C. The extract was filtered, and the concentration of NO_3^--N in the extract was analyzed by HPLC (SHIMADZU, HIC-6A, Kyoto, Japan) within 72 h to avoid the transformation of nitrate in the extract.

Perlite Analysis

A 5-g subsample of perlite from each cultivation pot was extracted with 50 ml of 2 M KCl and filtered. The NO_3^--N concentration in the extract was determined by diazotization after reduction of NO_3^--N to NO_2^--N with zinc powder[17]. The amount of NO_3^--N retained in perlite was calculated as N per 100 ml core (µmol N 100 ml^{-1}) and compared with the data of the NO_3^--N pool size in the forest soil where seeds of *L. triloba* were collected (Koyama, unpublished data). In the forest, 30 soil samples were collected from areas within a 30-cm radius from ten trunks of *L. triloba* in Mt. Ryuoh; this process was repeated five times during the 1998 growing season. A total of 150 soil samples were measured to determine NO_3^--N pool sizes in the forest.

Statistical Analysis

All statistical analyses were conducted using the statistical program SPSS 7.5.1[18]. Differences among NRAs or NO_3^--N concentrations in plants supplied with different concentrations of NO_3^--N were analyzed using a Kruskal–Wallis one-way analysis of variance. Multiple comparisons of mean values among treatments were performed by the sequential Bonferroni test[19] after the determination of pairwise P values by the Mann–Whitney test. In cases where multiple comparisons indicated that saturation had occurred in the relation between supplied NO_3^--N and NRA or NO_3^--N concentrations in the plants, Michaelis–Menten kinetics was applied for the relation of supplied NO_3^--N and plant NRA or NO_3^--N concentration as follows[20]:

$$v = S \times V_{max}/(S + Km)$$

where v is plant NRA or NO_3^--N concentration, S is the concentration of supplied NO_3^--N, Vmax is maximum value, and Km is the Michaelis constant. The two parameters, Vmax and Km, in the Michaelis–Menten kinetics were estimated by an Eadie–Hofstee plot (i.e., the relation of supplied NO_3^--N to supplied NO_3^--N/NRA)[21], and they were applied as initial values in the nonlinear regression analysis in SPSS. Spearman rank correlation coefficients were calculated to detect a relationship between NRA and NO_3^--N concentration in each of plant leaves and roots. Spearman rank correlation coefficients were also calculated to detect a relationship between leaves and roots for each of NRA and NO_3^--N concentration.

RESULTS

Plant NRA and NO_3^--N Concentration

Root NRA changed with NO_3^--N supply in the range from 0.071 to 1.79 mmol N l^{-1} supplied NO_3^--N (Fig. 1a); however, there was no significant difference between root NRA of individuals supplied with 1.79 and 3.57 mmol N l^{-1} NO_3^--N, indicating that root NRA had reached a plateau. The nonlinear regression analysis yielded values of Vmax = 0.46 (µmol N g dry wt^{-1} h^{-1}) and Km = 1.33 (mmol N l^{-1}) for the relationship between root NRA and NO_3^--N supply. In contrast, leaf NRA remained low even at the highest concentration of NO_3^--N, and there was no significant difference among treatments. Root NO_3^--N concentrations increased with NO_3^--N supply (Fig 1c); however, leaf NO_3^--N concentrations remained low with increased NO_3^--N supply (Fig 1d), even though the NO_3^--N concentrations in leaves were higher than in roots when the concentration of supplied NO_3^--N was lower than 0.071 mmol N l^{-1} ($p < 0.01$).

Comparisons of results between roots and leaves showed that there was no significant correlation between root NRA and leaf NRA or between root NO_3^--N concentration and leaf NO_3^--N concentration (Fig. 2). There was no significant correlation between leaf NO_3^--N concentration and leaf NRA, although root NRA was significantly correlated with root NO_3^--N concentration ($p < 0.01$) (Fig. 3).

Perlite NO_3^--N

The amount of NO_3^--N retained in perlite increased from 0 up to 154.26 µmol N 100 ml $perlite^{-1}$ and was significantly correlated with the NO_3^--N supply ($p < 0.001$) (Fig. 4a). Using the regression of supplied NO_3^--N to retained NO_3^--N in perlite, the perlite supplied with 2.06 mmol N l^{-1} NO_3^--N was equal to the maximum NO_3^--N pool size in the forest soil (79.47 µmol N 100 ml $soil^{-1}$, Fig. 4b).

DISCUSSION

Effects of NO_3^--N Supply on NO_3^--N Use by *L. triloba*

When the concentration of supplied NO_3^--N was lower than 1.79 mmol N l^{-1}, NRA in *L. triloba* roots increased with NO_3^--N supply (Fig. 1a). Because there was no significant difference between root NRA supplied with 1.79 mmol N l^{-1} and with 3.57 mmol N l^{-1}, it is likely that root NRA was saturated with 1.79 mmol N $l^{-1}$of NO_3^--N. Two possible explanations can be considered for the control of root NRA by NO_3^--N: (1) limited uptake of NO_3^--N and (2) limited induction of NR by NO_3^--N after it is taken up. The concentration of NO_3^--N in plant organs is the difference between increase of NO_3^--N by uptake and decrease of NO_3^--N by reduction, as plants do not synthesize NO_3^--N[11]; therefore, NO_3^--N concentration in plant organs must be less than or equal to the NO_3^--N absorbed by the plant. Nonetheless, root NO_3^--N concentrations continuously increased with NO_3^--N supply, showing no plateau (Fig. 1c). This indicates that the saturation of root NRA was not caused by the limited absorption of NO_3^--N, although there was a significant correlation between root NO_3^--N concentration and root NRA ($p < 0.01$) (Fig. 3a). This suggested that the NO_3^--N uptake by *L. triloba* corresponds to the NO_3^--N supply, even though the NO_3^--N utilization of this species did not correspond to the absorbed NO_3^--N when excess amounts of NO_3^--N were supplied.

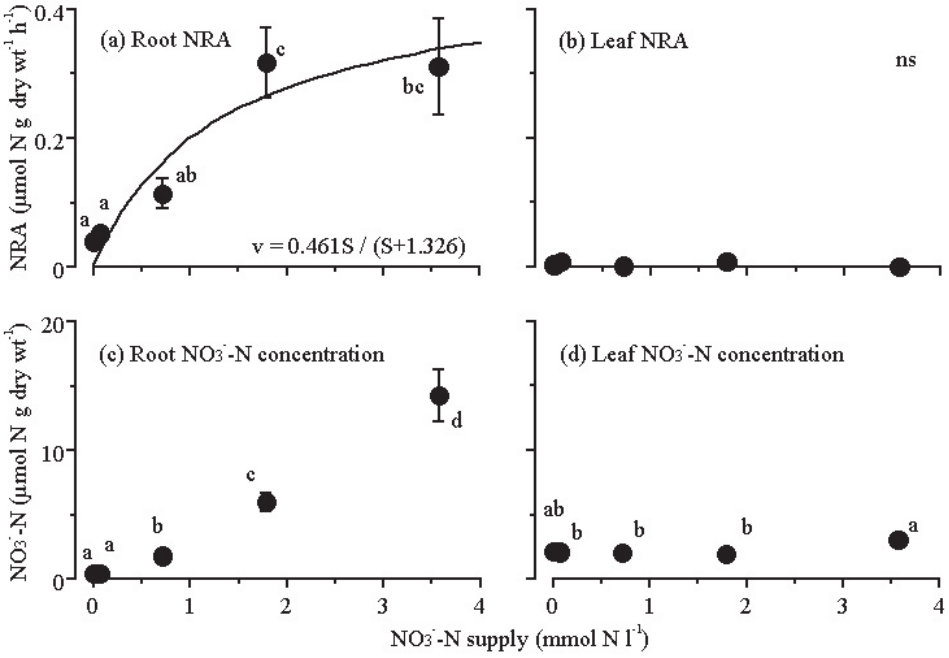

FIGURE 1. Effect of NO_3^--N supply on (a) root NRA (µmol N g dry wt^{-1} h^{-1}), (b) leaf NRA (µmol N g dry wt^{-1} h^{-1}), (c) root NO_3^--N concentration (µmol N g dry wt^{-1}), and (d) leaf NO_3^--N concentration (µmol N g dry wt^{-1}). The curved line shown in (a) shows the Michaelis–Menten kinetics. The bars show S.E.

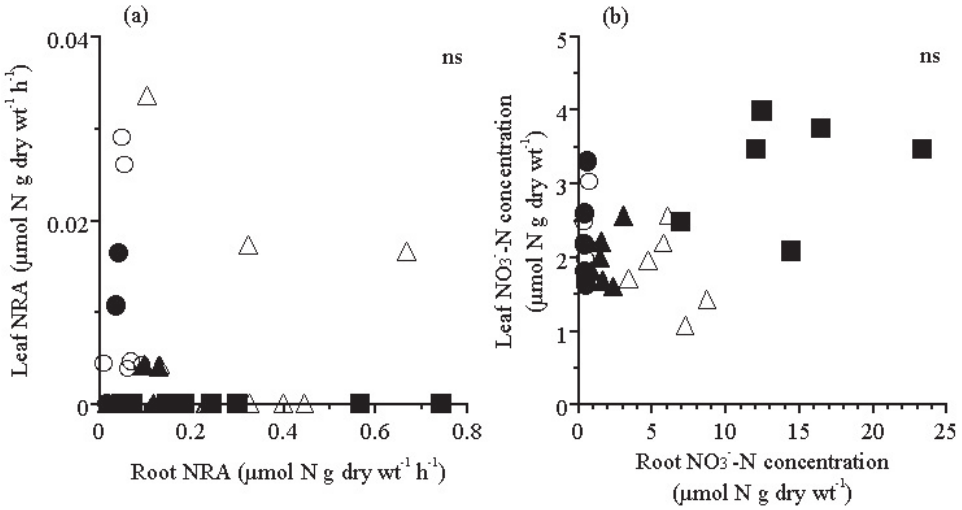

FIGURE 2. Relationship between (a) root NRA (μmol N g dry wt^{-1} h^{-1}) and leaf NRA (μmol N g dry wt^{-1} h^{-1}) and (b) root NO_3^--N concentration (μmol N g dry wt^{-1}) and leaf NO_3^--N concentration (μmol N g dry wt^{-1}). Concentrations of supplied NO_3^--N were 0 (●), 0.071 (○), 0.71 (▲), 1.79 (△), and 3.57 (■) mmol N l^{-1}.

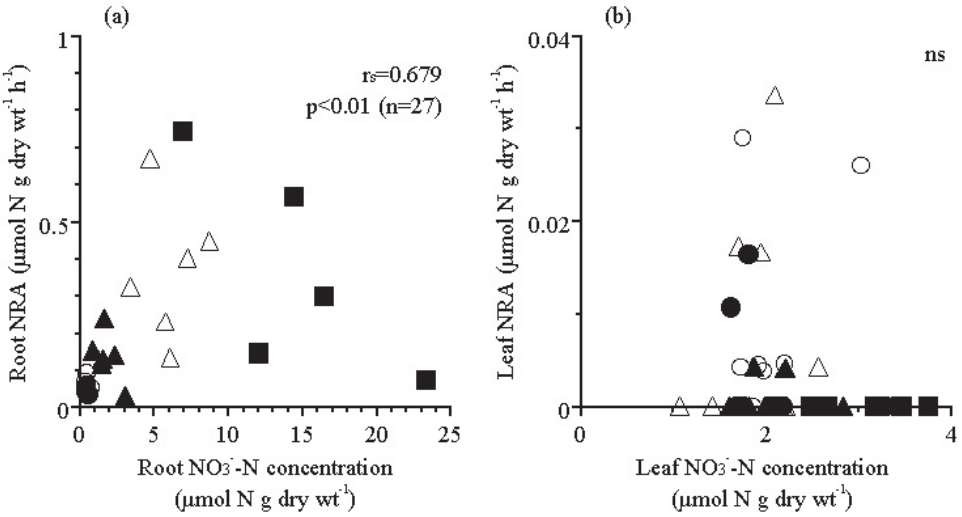

FIGURE 3. Relationship between (a) root NO_3^--N concentration (μmol N g dry wt^{-1}) and root NRA (μmol N g dry wt^{-1} h^{-1}) and (b) leaf NO_3^--N concentration (μmol N g dry wt^{-1}) and leaf NRA (μmol N g dry wt^{-1} h^{-1}). Concentrations of supplied NO_3^--N were 0 (●), 0.071 (○), 0.71 (▲), 1.79 (△) and 3.57 (■) mmol N l^{-1}.

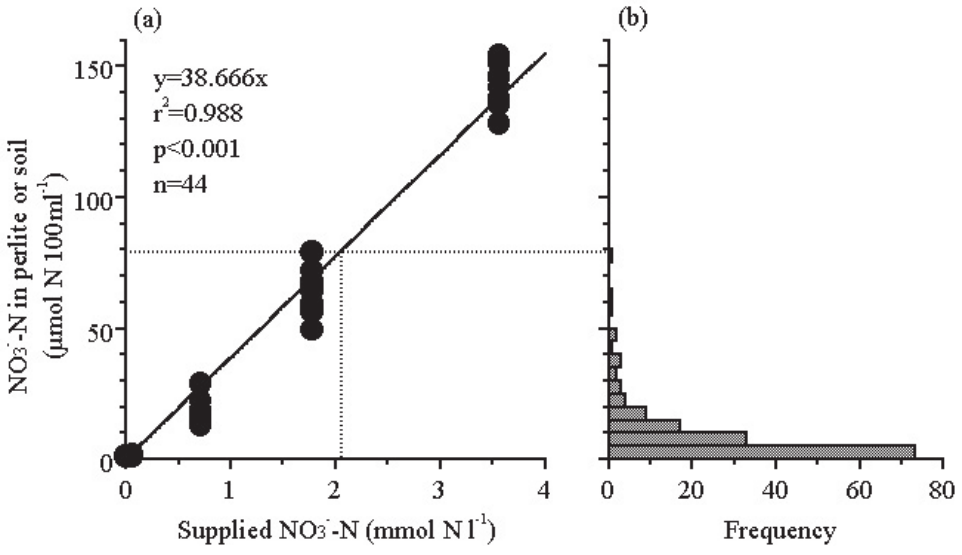

FIGURE 4. Comparison of NO_3^--N content in perlite and forest soil. (a) Relationship between NO_3^--N supply (mmol N l^{-1}) and amount of retained NO_3^--N in perlite (μmol N $100ml^{-1}$). (b) Frequency distribution for soil NO_3^--N pool size (μmol N 100 ml^{-1}) in the forest where the seeds were collected (Koyama, unpublished data). The dotted lines connecting figures indicated (1) the maximum of NO_3^--N pool size in forest soil and (2) the corresponding NO_3^--N supply to achieve that maximum.

On the other hand, there was no significant difference in leaf NRA supplied with different concentrations of NO_3^--N (Fig. 1b). Moreover, mean leaf NRA values were constantly lower than root NRA, irrespective of supplied NO_3^--N concentration ($p < 0.001$). Two reasons can be offered for very low NRA in leaves compared with roots: (1) the lack of enzyme induction in leaves and (2) the lack of NO_3^--N transportation from roots to leaves. The former reason, however, is not plausible because an investigation on *L. triloba* naturally grown in a conifer plantation showed that this species has NRA in its leaves, and the activity was approximately comparable to root NRA detected in this study (Koyama, unpublished data); therefore, it is obvious that *L. triloba* is able to induce NR in its leaves when NO_3^--N is transported to the leaves. Besides leaf NRA, leaf NO_3^--N concentrations also remained low even when a high concentration of NO_3^--N was supplied, and root NO_3^--N concentration showed no relationship with leaf NO_3^--N concentration (Figs. 1d and 2b). The lack of a significant correlation between leaf NRA and leaf NO_3^--N concentration can be ascribed to the narrower range of NRA and NO_3^--N concentrations in leaves than in roots (Fig. 3b); therefore, leaves of *L. triloba* may play only minor part in NO_3^--N use in the case of seedlings, and it may be because NO_3^--N absorbed by roots was not transported to the leaves. The transportation of NO_3^--N in plants and the allocation of NRA are influenced by factors such as specific property, light condition, external NO_3^--N availability, plant age, and/or temperature[7,8,22,23,24,25,26]. Specific differences and light conditions cannot explain the absence of (or very low) leaf NRA in the present study. It is because the same species showed foliar NRA in field investigations as stated above; and the light availability must be higher in the cultivation experiment than under the field conditions in the conifer plantation, though it is commonly accepted that the better light conditions provide plants an advantage in leaf NO_3^--N reduction[7,25]; however, further information is required to clarify the effects of other possible factors.

Moreover, when the concentrations of supplied NO_3^--N were lower than 0.071 mmol N l^{-1}, NO_3^--N, concentrations were significantly higher in leaves than in roots ($p < 0.01$), even though NRA was significantly lower in leaves than in roots across all levels of supplied NO_3^--N ($p < 0.01$). This result suggests that *L. triloba* has a storage pool of NO_3^--N in its leaves separate from the site of metabolism, and the NO_3^--N transported into the storage pool cannot be assimilated. It could, however, play a part in ionic and osmotic balance in the cells[27].

Estimation of NO_3^--N Availability in Forest Soil to NO_3^--N Use by *L. triloba*

Because the supplied solution (200 ml) overflowed the seedling receptacles, the NO_3^--N available to *L. triloba* was equivalent not to the total amount of added NO_3^--N but to the amount of NO_3^--N retained in perlite. Among the treatments, however, the amount of NO_3^--N retained in perlite was significantly correlated with the concentration of supplied NO_3^--N ($p < 0.001$) (Fig. 4a); therefore, the range of NO_3^--N pool size in forest soils (from 0 to 79.47 µmol N 100 ml $soil^{-1}$) was equivalent to the amount of NO_3^--N retained in perlite supplied with NO_3^--N of 0 to 2.06 mmol N l^{-1} from the regression. The substitution of the maximum NO_3^--N pool size in forest soil (namely, supply of 2.06 mmol N l^{-1} NO_3^--N) for the Michaelis–Menten kinetics gives 0.28 µmol N g dry wt^{-1} h^{-1} in NRA, which is equivalent to 60.8% of maximum value (0.46 µmol N g dry wt^{-1} h^{-1}). Because there was no significant difference between the seedlings supplied with 1.79 mmol N l^{-1} NO_3^--N and with 3.57 mmol N l^{-1} NO_3^--N (Fig. 1a), root NRA might almost reach the plateau when 2.06 mmol N l^{-1} NO_3^--N (that is equivalent to the maximum NO_3^--N pool size in forest soils) was supplied; however, as the frequency distribution for soil NO_3^--N pool size was positively skewed (Fig. 4b), 90% of forest soils had a smaller NO_3^--N pool size than perlite supplied with 0.71 mmol N l^{-1} NO_3^--N. When NO_3^--N availability is in this range, roots of *L. triloba* are likely have a value of NRA lower than 0.16 µmol N g dry wt^{-1} h^{-1} (35.0% of maximum), assuming the preceding Michaelis–Menten kinetics apply. Moreover, root NRA increased with NO_3^--N supply across this range, suggesting that NO_3^--N availability is the limiting factor for NO_3^--N assimilation by *L. triloba* grown in forest soils. These comparisons between NO_3^--N retained in perlite and NO_3^--N in forest soils suggest that available NO_3^--N in forest soils is below the saturation concentration for *L. triloba*.

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REFERENCES

1. Bobbink, R., Hornung, M., and Roelofs, J.G.M. (1998) The effects of air-borne nitrogen pollutants on species diversity in natural and semi-natural European vegetation. *J. Ecol.* **86**, 717–738.
2. Vitousek, P.M., Aber, J.D., Howarth, R.H., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., and Tilman, D.G. (1997) Human alteration of the global nitrogen cycle: source and consequences. *Ecol. Appl.* **7**, 737–750.
3. Zak, D.R. and Pregitzer, K.S. (1988) Nitrate assimilation by herbaceous ground flora in late successional forests. *J. Ecol.* **76**, 537–546.
4. Rothstein, D.E., Zak, D.R., and Pregitzer, K.S. (1996) Nitrate deposition in northern hardwood forests and the nitrogen metabolism of *Acer saccharum* marsh. *Oecologia* **108**, 338–344.
5. Rothstein, D.E. (2000) Spring ephemeral herbs and nitrogen cycling in a northern hardwood forest: an experimental test of the vernal dam hypothesis. *Oecologia* **124**, 446–453.
6. Haynes, R.J. and Goh, K.M. (1978) Ammonium and nitrate nutrition of plants. *Biol. Rev.* **53**, 465–510.
7. Pate, J.S. (1983) Patterns of nitrogen metabolism in higher plants and their ecological significance. In *Nitrogen as an Ecological Factor*. Lee, J.A., McNeill, S., and Rorison, I.H., Eds. Blackwell Scientific Publications, Oxford. pp. 225–255.
8. Haynes, R.J. (1986) Uptake and assimilation of mineral nitrogen by plants. In *Mineral Nitrogen in the Plant–Soil System*. Kozlowski T.T., Ed. Academic Press, London. pp. 303–378.

9. Beevers, L. and Hageman, R.H. (1969) Nitrate reduction in higher plants. *Ann. Rev. Plant Physiol.* **20**, 495–522.
10. Campbell, W.H. (1988) Nitrate reductase and its role in nitrate assimilation in plants. *Physiol. Plant.* **74**, 214–219.
11. Melzer, A., Gebauer, G., and Rehder, H. (1984) Nitrate content and nitrate reductase activity in *Rumex obtusifolius*. 2. Responses to nitrate starvation and nitrogen fertilization. *Oecologia* **63**, 380–385.
12. Hirobe, M., Tokuchi, N., and Iwatsubo, G. (1998) Spatial variability of soil nitrogen transformation patterns along a forest slope in a *Cryptomeria japonica* D. Don plantation. *Eur. J. Soil. Biol.* **34**, 123–131.
13. Jaworski, E.G. (1971) Nitrate reductase assay in intact plant tissues. *Biochem. Biophys. Res. Commun.* **43**, 1274–1279.
14. Gebauer, G., Melzer, A., and Rehder, H. (1984) Nitrate content and nitrate reductase activity in *Rumex obtusifolius*. 1. Differences in organs and diurnal changes. *Oecologia* **63**, 136–142.
15. Högberg, P., Granstrom, A., Johansson, T., Lundmark, T.A., and Näsholm, T. (1986) Plant nitrate reductase activity as an indicator of availability of nitrate in forest soils. *Can. J. For. Res.* **16**, 1165–1169.
16. Gebauer, G., Rehder, H., and Wollenweber, B. (1988) Nitrate, nitrate reduction and organic nitrogen in plants from different ecological and taxonomic groups of Central Europe. *Oecologia* **75**, 371–385.
17. Keeney, D.R. and Nelson, D.W. (1982) Nitrogen: inorganic forms. In *Methods of Soil Analysis. Part 2*. Page, A.L., Miller, R.H., and Keeney, D.R., Eds. American Society of Agronomy/Soil Science Society of America, Madison, WI.. pp. 643–698.
18. SPSS (1996) SPSS for Windows. SPSS Inc., Chicago.
19. Rice, W.R. (1988) Analyzing tables of statistical tests. *Evolution* **43**, 223–225.
20. King, B.J., Siddiqui, M.Y., and Glass, A.D.M. (1992) Studies of uptake of nitrate in barley. V. Estimation of root cytoplastic nitrate concentration using nitrate reductase activity: implication for nitrate influx. *Plant Physiol.* **99**, 1582–1589.
21. Metzler, D.E. (2001) Enzymes: the catalysts of cells. In *Biochemistry: The Chemical Reactions of Living Cells*. Academic Press, San Diego. pp. 455–503.
22. Andrews, M. (1986) The partitioning of nitrate assimilation between root and shoot of higher plants. *Plant Cell Environ.* **9**, 511–519.
23. Fredeen, A.L., Griffin, K., and Field, C.B. (1991) Effects of light quantity and quality and soil nitrogen status on nitrate reductase activity in rainforest species of genus Piper. *Oecologia* **86**, 441–446.
24. Stewart, G.R., Joly, C.A., and Smirnoff, N. (1992) Partitioning of inorganic nitrogen assimilation between the roots and shoots of cerrado and forest trees of contrasting plant communities of South East Brasil. *Oecologia* **91**, 511–517.
25. Gojon, A., Plassard, C., and Bussi, C. (1994) Root/shoot distribution of NO_3- assimilation in herbaceous and woody species. In *A Whole Plant Perspective on Carbon-Nitrogen Interactions*. Roy, J. and Garnier, E., Eds. Academic Publishing, The Hague. pp. 131–147.
26. Peuke, A.D. and Kaiser, W.M. (1996) Nitrate or ammonium uptake and transport, and rapid regulation of nitrate reduction in higher plants. In *Progress in Botany 57: Physiology*. Behnke, H-D., Lüttge, U., Esser, K., Kadereit, J.W., and Runge, M., Eds. Springer–Verlag, Berlin. pp. 93–113.
27. Martinoia, E., Heck, U., and Wiemken, A. (1981) Vacuoles as storage compartments for nitrate in barley leaves. *Nature* **289**, 292–294.

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