

Nutrient foraging via physiological and morphological plasticity in three plant species

Lixin Wang, Paul P. Mou, and Robert H. Jones

Abstract: Physiological and morphological plasticity of roots enhance plant nutrient uptake in spatiotemporally heterogeneous soil environments. We examined these two types of plasticity using three plant species (*Solidago altissima* (L.) Raf., *Pinus taeda* L., and *Liquidambar styraciflua* L.). We grew plants in pots (one plant per pot) with equal quantity of fertilizer applied either evenly over the pot surface (H) or on one-quarter of the pot surface (T). A high-concentration ^{15}N -labeled ammonium nitrate solution was injected twice over 48 h before harvest at a random location in H pots, and in either unfertilized or fertilized portion of T pots. Physiological plasticity of N uptake was observed in *S. altissima* and *L. styraciflua*. The highest ^{15}N uptake rate for *L. styraciflua* occurred in H pots (medium level), and that for *S. altissima* occurred in fertilized portions of T pots (rich level). When low-concentration ^{15}N was added to *S. altissima*, no differences in uptake were noted among treatments, possibly because of interroot competition. In *S. altissima* and *P. taeda*, either morphological or physiological plasticity was strong. In *L. styraciflua*, both types of plasticity were strong. Total ^{15}N uptake was enhanced when ^{15}N was added to the fertilized patches. Physiological plasticity contributed >70% of enhanced ^{15}N uptake in *S. altissima* and *L. styraciflua*.

Résumé : La plasticité physiologique et morphologique des racines augmente l'absorption d'éléments nutritifs chez les plantes vivant dans des sols spatiotemporellement hétérogènes. Les auteurs ont examiné ces deux types de plasticité en utilisant trois espèces végétales (*Solidago altissima* (L.) Raf., *Pinus taeda* L. et *Liquidambar styraciflua* L.). Ils ont fait pousser les plantes en pots (une plante par pot) avec une quantité égale de fertilisant appliquée ou bien uniformément sur la surface du pot (H) ou bien sur un quart de la surface du pot (T). Une solution concentrée de nitrate d'ammonium marquée à l'azote radioactif (^{15}N) a été injectée deux fois dans les 48 heures précédant la récolte à des endroits choisis au hasard dans les pots H et dans la portion fertilisée ou la portion non fertilisée des pots T. Une plasticité physiologique de l'absorption de N a été observée chez *S. altissima* et *L. styraciflua*. Le plus haut taux d'absorption de ^{15}N pour *L. styraciflua* est survenu dans les pots H (niveau intermédiaire) alors que celui de *S. altissima* est survenu dans la portion fertilisée des pots T (niveau riche). Lorsqu'une faible concentration de ^{15}N a été ajoutée au *S. altissima*, aucune différence d'absorption des nutriments n'a été notée entre les traitements, possiblement à cause de la compétition entre les racines. Chez *S. altissima* et *P. taeda*, soit la plasticité morphologique soit la plasticité physiologique était importante. Chez *L. styraciflua*, les deux types de plasticité étaient importants. L'absorption totale de ^{15}N était meilleure quand ^{15}N était ajouté aux endroits fertilisés. La plasticité physiologique a contribué à plus de 70 % de l'augmentation de l'absorption de ^{15}N chez *S. altissima* et *L. styraciflua*.

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Introduction

In soil, patchiness has been demonstrated for nutrients (Jackson and Caldwell 1993a; Lister et al. 2000), water availability (Guo et al. 2002; Snyder and Williams 2003), soil organic matter and pH (Jackson and Caldwell 1993b), and soil texture and structure (Brady and Weil 1999). Patches vary in scale, distribution, extent, and number (Fitter 1994). Furthermore, patterns of patchiness vary from one community to

the next and change during succession (Mou et al. 1993; Guo et al. 2002). Plant root systems respond to soil heterogeneity via plasticity in morphology, physiology, demography, and mycorrhizas (Jackson et al. 1990; Pregitzer et al. 1993; Smith and Read 1997). These types of foraging plasticity may be adaptive for different spatiotemporal patterns of resource heterogeneity (Campbell et al. 1991; Hutchings and De Kroon 1994; Wijesinghe et al. 2001).

Many have investigated morphological plasticity of plant root systems, which is defined as an increase in the local formation and growth of lateral roots in response to local resource enrichment (Hutchings and De Kroon 1994). Plant roots tend to proliferate in nutrient-enriched patches, especially when the overall resource level is low (Crick and Grime 1987; Eissenstat and Caldwell 1988; Einsmann et al. 1999). The degree of response varies across species (Robinson 1994; Mou et al. 1995; Rajaniemi and Reynolds 2004).

We define physiological plasticity as variation of nutrient uptake kinetics or nutrient inflow due to preconditioning of the roots to different nutrient regimes (Jackson et al. 1990).

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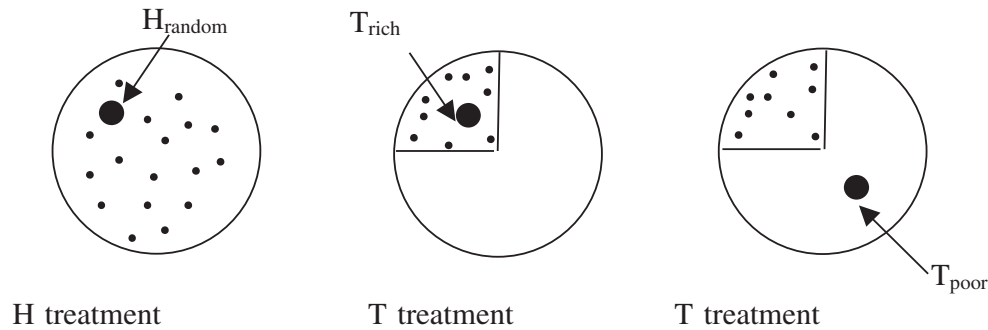
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Fig. 1. An illustration of spatial arrangements of slow-release fertilizer in homogeneous (H) and heterogeneous (T) treatments, and the locations of ^{15}N solution application (indicated by the large black dots) to test for uptake rates of roots developed in three different nutrient levels (medium (H_{random}), high (T_{rich}), and low (T_{poor})).



Under this definition, a plant species is plastic when roots exposed to different previous nutrient regimes (e.g., high versus low availability) show different uptake kinetics when exposed to the same experimentally controlled nutrient level. Several previous studies have demonstrated that physiological plasticity, defined in this way, exists (Drew and Saker 1978; Barber and Anghinoni 1980; Jackson et al. 1990). We examined physiological plasticity for four plant species (*Erechtites hieracifolia* (L.) Raf., *Liquidambar styraciflua* L., *Pinus taeda* L., and *Solidago altissima* (L.) Raf.) in a greenhouse experiment by applying a pulse of ^{15}N -labeled fertilizer (Bliss 2001). Uptake rates per unit fine root mass were greater for roots developed in nutrient-poor patches than for those in nutrient-rich patches. We found this result interesting and worthy of follow-up study because it differed from findings in Jackson et al. (1990), which showed that roots of several desert plant species developed in nutrient-rich patches had higher uptake rates than root developed in nutrient-poor patches.

The benefits of morphological and physiological plasticity may vary according to the temporal nature of nutrient patchiness. When soil patches persist for longer periods of time, morphological plasticity may be pervasive and effective in enhancing plant nutrient uptake (Birch and Hutchings 1994). In contrast, physiological plasticity may be more effective and efficient when soil nutrient patchiness shifts more frequently in time and space (Robinson et al. 1994; Hodge 2004). Derner and Briske (1999) suggested that morphological and physiological root plasticity may represent complementary rather than alternative foraging strategies. However, few studies have been conducted to examine morphological and physiological plasticity together at the whole-plant level (van Vuuren et al. 1996; Robinson 2001), and the relationship between the two types of plasticity, to our knowledge, has not been experimentally examined. Relationships among different types of nutrient-foraging plasticity in roots may have important implications for predicting outcomes of competitive interactions or responses to changes in environmental conditions (Hodge 2004).

To examine relationships between root foraging traits, it will be important to consider mycorrhizal formation. More than 90% of vascular plants have the potential to form mutualistic mycorrhizal associations that enhance plant nutrient uptake (Fitter 1977; Trappe 1977; Ames et al. 1983, 1984; Johansen et al. 1992, 1993). Mycorrhizal colonization may affect root morphological plasticity (Cui and Caldwell 1996),

and this effect may vary with respect to types of mycorrhizas (Pregitzer et al. 2002).

In this study, we used a stable-isotope approach to examine both physiological and morphological plasticity in root nutrient foraging for three plant species. Plants were grown in pots with patchy or homogeneous nutrient conditions (patches) created by slow-release inorganic fertilizer. Soil patches were pulsed with ^{15}N -labeled nutrient, and then whole plants were harvested. By evaluating whole-plant ^{15}N uptake and scaling this value to the density of roots in the ^{15}N -dosed soil patches, both physiological and morphological plasticity can be quantified.

The main objectives were (1) to determine whether physiological and morphological plasticity exist in the experimental plant species; (2) to examine whether major differences exist in root foraging plasticity among the species, particularly in physiological plasticity; and (3) to begin exploring relationships between physiological plasticity and morphological plasticity of root foraging. Using results from previous studies, we predicted that (1) both physiological and morphological plasticity in nitrogen (N) foraging exist in all plant species; (2) the degree of physiological and morphological plasticity differs across species; and (3) if the three species in this study differ in morphological plasticity, they would also differ in physiological plasticity, but in the reverse order.

Materials and methods

Experiment setup

The experiment was conducted in an environmentally controlled greenhouse at the University of North Carolina at Greensboro from July 2002 to June 2003. We used the following species: *L. styraciflua* (sweetgum), a deciduous tree, *P. taeda* (loblolly pine), an evergreen tree, and *S. altissima* (goldenrod), a perennial herb. The three species are widely distributed throughout the coastal plain of the southeastern United States (Miller et al. 1995). We collected seeds of *S. altissima* from US Department of Energy's Savannah River Site in South Carolina, and purchased the seeds of the two tree species from a commercial seed company.

Tree seeds were stratified under 4 °C for 1–3 months (to enhance germination rate) before being sown in germination seed trays filled with commercial potting soil (Sta-green, manufactured for Spectrum Group, Division of United In-

dustries Corporation, St. Louis, Missouri). When the height of the tree seedlings reached 5 cm, they were transplanted into plastic pots (28 cm in height and 15.5 cm in radius) with one seedling per pot (Fig. 1). The pots were filled with 19 L of fine construction-grade sand with a very low nutrient content. Seeds of *S. altissima* were germinated with the same procedure. They were transplanted to the 19 L pots when the seedling crowns reached 3 cm in diameter. The root systems were small when the seedlings were transplanted, and the status of mycorrhizal infection was difficult to judge. Twenty millilitres of 1:1000 (*mlm*) fertilizer (N–P–K, 16:5:16) solution (0.02 g fertilizer per pot each time, which is equivalent to 0.11 g N, 0.07 g P, and 0.09 g K per square metre) was applied twice to each plant during the first week after the transplantation to assist seedling establishment. A total of 120 plants were planted into 120 pots, with 40 pots per species.

Plants were randomly assigned to two nutrient treatments: homogenous (H hereafter) and heterogeneous (T hereafter). In each H pot, 5 g (equivalent to 27.58 g N, 16.55 g P, and 22.05 K per square metre) of slow-release fertilizer (15:9:12, N–P–K plus micronutrients; the N–P–K forms of this fertilizer are NH_4 , NO_3 , P_2O_5 , and K_2O , respectively, Osmocote plus, the Scotts Co., Marysville, Ohio) was evenly applied to the soil surface. While in each T pot, application of the same amount of fertilizer was concentrated in one-quarter of the pot soil surface (Fig. 1, Mou et al. 1997). This fertilization intensity created a mineral-nutrient condition comparable to that in field conditions of the coastal plain of the southeastern United States. The lateral movement of nutrients in pot soil is minimal (Einsmann et al. 1999). For each species, 20 pots received the H treatment and 20 pots received the T treatment. During the growing period, the plants were misted daily to avoid water stress. The greenhouse temperature was set at 23 °C for daytime and 16 °C for nighttime, with a photoperiod of 14 h to mimic the growing season climate at the native sites of the plants (South Carolina State Climatology Office, South Carolina Department of Natural Resources, Columbia).

¹⁵N treatment, sample collection, and sample process

By the end of growing period (12–16 weeks for *S. altissima*, 30–40 weeks for *P. taeda* and *L. styraciflua*), healthy plants with sufficient sizes were selected to receive ¹⁵N treatment to test root physiological plasticity. The individuals of *S. altissima* received ¹⁵N treatment when the rosettes roughly covered the pot soil surface, while the individuals of *L. styraciflua* and *P. taeda* received the treatment when the stem heights reached 40–45 cm. In total, 54 plants received ¹⁵N treatment.

After a 48 h drying period (i.e., no irrigation), a 50 mL syringe with a 12 cm needle was used to inject 20 mL of 5 atom % ¹⁵N dual labeled ammonium nitrate solution (0.58 mol·L⁻¹ of N) twice to the same location with a 24 h interval in 44 plants (*S. altissima*: 14, *L. styraciflua*: 16, and *P. taeda*: 14). In each pot, there was just one injection location that was in the center of either the fertilized quarter (T_{rich} : high nutrient level) or the unfertilized quarter (T_{poor} : low nutrient level) of T pots, and in the center of a randomly selected quarter (H_{random} : medium nutrient level) for H pots (Fig. 1). Injection was carried out carefully to assure equal amounts of application per unit depth increment from the soil surface

to the depth of 12 cm. The injected solution formed a treated soil column ~15 cm in depth and 1–1.5 cm in radius after the diffusion. Ten additional *S. altissima* plants received dual ¹⁵N labeled ammonium nitrate with a lower N concentration (0.1 mol·L⁻¹ of N) to examine whether lower dose application would yield different results because of strong interroot competition (i.e., we assumed that the higher dose was sufficiently high to overwhelm strongly competitive diffusion gradients created by adjacent roots). For the low-dose application, all procedures were the same as those for the high-dose application. We mixed commercial food dye (active ingredients: propylene glycol and propylparaben) in the solution for easy identification of the treated soil columns and roots when they were harvested.

Each plant was harvested 24 h after the second injection. The plant top was cut at the soil surface. The leaves and stems were separated and then oven-dried at 65 °C to constant mass for dry mass evaluation. The ¹⁵N-treated column (soil and roots) in each pot was taken out with a 5 cm diameter edge-sharpened metal tube. The harvested soil plug (which included the treated red-colored column) was stored at 2–4 °C in a sealed plastic bag before processing. The rest of the plant's root system was harvested from each soil quarter by washing the soil–root mixture over a 4 mm mesh, and then the roots were oven-dried at 65 °C to constant mass for dry mass evaluation. Woody species produced a thick taproot that was harvested, dried, and weighed, but not used to calculate spatial distribution of roots among pot quarters.

Soil plugs were processed within 12 h after the harvest. The dyed soil and root column was carefully separated from nondyed soil. The fine roots (≤ 1 mm in diameter) and the coarse roots in the soil plug were sorted out and separated, and the dyed fine roots were carefully separated from the other root material. The length of the dyed fine roots of each soil plug was estimated with the grid-intersection method (Böhm 1979). The fine roots were then oven-dried to constant mass at 65 °C and weighed to calculate specific root length (SRL = root length (cm) / root mass (mg), Böhm 1979). The fine roots were further subsampled and ground for ¹⁵N analysis. Both fine and coarse roots from each soil quarter were also sorted out and separated, oven-dried at 65 °C to constant mass, weighed, subsampled, and ashed. The ash-free masses of fine roots and coarse roots in each soil quarter were determined. Roots, leaves, and stems from each plant were subsampled for ¹⁵N analysis. To estimate background ¹⁵N levels, we also collected fine roots, coarse roots (for trees), stem (for trees), and leaves from a random selection of 13 plants not subjected to ¹⁵N injection. All plant tissue samples were sent to the Stable Isotope Mass Spectrometry laboratory at North Carolina State University for ¹⁵N analysis.

Calculations

Total ¹⁵N uptake per plant was computed. We first subtracted tissue (root, leaf, and stem) ¹⁵N concentrations of control plants from ¹⁵N concentrations of corresponding parts of injected plants. This calculation yielded tissue ¹⁵N concentration differences ($T^{15}\text{NCD}$) for leaf, stem, coarse roots, and fine roots. Total ¹⁵N uptake of each plant was calculated as follows:

$$\text{Total } ^{15}\text{N uptake} = \sum (T^{15}\text{NCD}_i \times \text{tissue masses}_i)$$

Table 1. Results of two-way ANOVA of plant total ^{15}N uptake, fine root ^{15}N uptake rate (per unit fine root length and per unit fine root mass), and specific root length (SRL) with species and treatment (nutrient levels that ^{15}N solution was applied to) as main effects.

Source	df	<i>F</i> value			
		Total ^{15}N uptake	^{15}N uptake per unit fine root mass	^{15}N uptake per unit fine root length	SRL
S	2	16.37****	7.97**	35.21****	35.21****
T	2	12.78***	0.93	2.39	4.75*
S \times T	4	1.06	4.01**	2.28	1.21
Error	28				

Note: S, species; T, treatment. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

where i refers to types of tissues (i.e., leaf, stem, coarse roots, and fine roots). ^{15}N uptake rate was then calculated as total ^{15}N uptake divided by fine root length or mass in the corresponding soil plug (Appendix A).

N uptake physiological plasticity was assessed by comparing ^{15}N uptake rate (per unit fine root mass or length) among roots in the soil plugs from three nutrient levels (T_{rich} , H_{random} , and T_{poor}). A significant difference in any pair indicates physiological plasticity in nutrient foraging (i.e., roots developed in different nutrient levels having different uptake rates when exposed to same amount of N solution). To estimate the degree of physiological plasticity for a species, P_{phys} , we determined the maximum mean difference in ^{15}N uptake per unit fine root mass among all three nutrient regimes (T_{rich} , H_{random} , and T_{poor}). Greater P_{phys} values indicate greater physiological plasticity.

Fine root morphological plasticity was estimated using the relative fine root mass difference (RFRMD) (Mou et al. 1997). For the T treatment, $\text{RFRMD} = ((\text{fine root mass in } T_{\text{rich}} \text{ quarter} - \text{fine root mass in } T_{\text{poor}} \text{ quarter}) / \text{total fine root mass in the pot}) \times 100$, and for the H treatment, $\text{RFRMD} = ((\text{fine root mass difference in two opposite quarters randomly chosen}) / \text{total fine root mass in the pot}) \times 100$. RFRMD for H pots is expected to be 0. In the T pots (RFRMDT hereafter) values greater than 0 indicate morphological plasticity.

We developed a computation to estimate relative contribution of each type of plasticity to N-uptake increases in fertilized patches. ^{15}N uptake rate (mass) in the poor patches was used as a base value. The base value was multiplied by the root mass difference between the poor and the medium or rich plugs to achieve the amount of ^{15}N uptake purely due to morphological plasticity. Then this value was subtracted from the total ^{15}N uptake increase (total ^{15}N uptake in medium or rich patches – total ^{15}N uptake in poor patch) to yield ^{15}N uptake due to physiological plasticity. The contribution of each type of plasticity was divided by the total ^{15}N uptake increase and expressed as a percentage.

Data analyses

Data from seven plants were irregular: two plants had too few roots (<0.0003 g) in the dosed plug to give meaningful results (one *L. styraciflua* in T_{poor} and one *S. altissima* in H), and five plants showed evidence that the fertilizer on the surface or below the soil moved away from the fertilized quarter as a result of pot tilting or sampling error, resulting in RFRMDT values smaller than 0 (one *P. taeda* in T_{rich} , one *P. taeda* in T_{poor} , and one *S. altissima* in T_{rich}) or in root mass in one-quarter being much more than that in the opposite

quarter in an H pot (one *L. styraciflua* and one *S. altissima*). We noted that in tilted pots, water ran across the soil surface and collected on the lower side of the pot. These seven data points were removed from the data set. Data of the remaining 47 plants, including 10 individuals of *S. altissima* dosed with a lower N concentration, were used in the data analyses.

Before conducting ANOVA, the ^{15}N uptake per unit fine root mass or length and total ^{15}N uptake data were tested by Levene's test to examine the equal variance requirement. If the data were heteroscedastic, they were log transformed, and the log-transformed data were used in analysis if assumptions of equal variance and normal distribution were met. RFRMD and SRL in all three species met the assumptions, and ANOVA was performed on nontransformed data.

N uptake physiological plasticity can be assessed using either root length or root mass data. However, analysis based upon root length can be confounded if root diameters vary across nutrient levels, and comparison among species becomes obscure if root length per unit of root mass is species specific (SRL). To determine whether root length or root mass was more appropriate for assessing physiological plasticity, two-way ANOVA (PROC GLM, SAS Institute Inc. 2000) was used to examine whether treatment, species, or a treatment \times species interaction significantly affected SRL. We found significant treatment and species effects (Table 1), and therefore, we chose to assess physiological plasticity using root mass instead of root length data.

To examine whether the three species exhibit physiological plasticity, two-way ANOVA (PROC GLM, SAS Institute Inc. 2000) was performed to compare ^{15}N uptake rates (mass) with species and treatment (T_{rich} , H_{random} , and T_{poor}) as main effects. Significant treatment or treatment \times species interactions were taken as evidence for physiological plasticity for at least one species, and if this evidence was found, within-species one-way ANOVA (PROC GLM, SAS Institute Inc. 2000) followed, with multiple comparisons tests to determine which of the nutrient treatments led to the greatest and least uptake rates. The differences between the greatest and least uptake rate were then used as an index (P_{phys}) of physiological plasticity. The contrast option of GLM procedure in SAS was performed on P_{phys} to examine species difference in the degree of physiological plasticity. For the individuals of *S. altissima* dosed with $0.1 \text{ mol}\cdot\text{L}^{-1}$ N solution, one-way ANOVA was used to examine treatment effects.

RFRMD values of each species were analyzed with a one-sample t test for each of the treatments (H and T). Morphological plasticity is revealed if RFRMD is not significantly

Table 2. Results of one-way ANOVA and multiple comparisons of plant ^{15}N uptake rate per unit fine root mass of each species for roots developed in three different nutrient levels.

Species	df	F	p	Avg. uptake rate (mg/g)		
				T _{rich}	H _{random}	T _{poor}
<i>L. styraciflua</i>	2, 11	5.57	0.0213	1.35a	4.63b	2.19ab
<i>P. taeda</i>	2, 9	0.26	0.7770	1.53a	1.21a	0.99a
<i>S. altissima</i>	2, 8	8.97	0.0091	4.33a	1.79b	1.71a

Note: T_{rich}, high nutrient level; H_{random}, medium nutrient level; T_{poor}, low nutrient level. Means within a species with the same letter are not significant different at the 0.05 level.

different from 0 in H pots, but significantly greater than 0 in T pots (Mou et al. 1997). To compare species difference in morphological plasticity, RFRMDT values of the three species were examined using one-way ANOVA.

Mycorrhizas survey

All three species reportedly form mycorrhizas: *P. taeda* forms ectomycorrhizas (EM) and the other two species form arbuscular mycorrhizas (AM). We surveyed mycorrhizal colonization rates in root samples from the pot plants and from the plants in the field to examine whether there is any difference in mycorrhizal colonization. EM colonization was visually examined under a dissecting microscope for samples each consisting of at least 50 root tips ($n = 3$ for field samples, each comprised a combination of four 10 cm diameter by 25 cm deep soil cores, and $n = 4$ for pot samples, each comprised root tips randomly picked from harvested roots). The presence of AM was identified using a stain technique following Sylvia (1994) ($n = 5$ and 8 in field plots for *L. styraciflua* and *S. altissima*, respectively, and $n = 11$ for each of the two species in pots). AM and EM colonization rates were determined using the grid line intersection method (Giovannetti and Mosse 1980). The colonization rate was calculated by the ratio of the number of mycorrhizal-colonized roots encountered at grid line intersections and the total number of roots encountered at grid line intersections.

Results

Physiological plasticity

Two-way ANOVA revealed a significant species effect, an insignificant treatment effect on ^{15}N uptake rate per unit fine root mass, and a significant interaction between species and treatment (Table 1). One-way ANOVA was therefore performed for each species to further examine ^{15}N uptake rate across treatments. *Solidago altissima* and *L. styraciflua* had significantly different ^{15}N uptake rates across the three nutrient levels (treatment), while *P. taeda* did not (Table 2). Multiple comparisons showed that ^{15}N uptake rate of *S. altissima* in the T_{rich} nutrient level was significantly higher than that in the H_{random} and T_{poor} nutrients levels, and ^{15}N uptake rate was higher, but not significantly, in H_{random} than in T_{poor} (Fig. 2, Table 2). ^{15}N uptake rate of *L. styraciflua* in the H_{random} nutrient level was significantly higher than that in the T_{rich} nutrient level, and ^{15}N uptake rate in T_{poor} was not significantly different from that in H_{random} and T_{rich} (Fig. 2, Table 2). ^{15}N uptake rates of *S. altissima* across three nutrient levels were not

significantly different when they were dosed with 0.1 mol·L⁻¹ N solution ($p = 0.167$, Fig. 2).

We decided, using our working definition of P_{phys} , that P_{phys} of *S. altissima* was the ^{15}N uptake rate difference between the T_{rich} and T_{poor} nutrient levels; P_{phys} for *L. styraciflua* was the ^{15}N uptake rate difference between the H_{random} and T_{rich} nutrient levels; and P_{phys} for *P. taeda* was the ^{15}N uptake rate difference between the T_{rich} and T_{poor} nutrient levels, though the later two were not significantly different from each other. The contrast option of GLM procedure indicated significant differences in P_{phys} between *S. altissima* and *P. taeda* and between *L. styraciflua* and *P. taeda* and a nonsignificant difference between *S. altissima* and *L. styraciflua*.

Morphological plasticity

All three species exhibited morphological plasticity. RFRMDs in each species were significantly greater than zero in T pots but not so in H pots (Table 3). Cross-species comparison of RFRMDT revealed a significant difference ($p < 0.006$). Multiple comparisons (Fisher's least significant difference test) among the species showed that RFRMDT values were significantly greater in *L. styraciflua* than in *S. altissima* ($p < 0.05$). The RFRMDT differences between *L. styraciflua* and *P. taeda* and between *S. altissima* and *P. taeda* were not significant. In this experiment, *L. styraciflua* had the greatest RFRMDT value, followed by *P. taeda* and then *S. altissima*.

Total ^{15}N uptake and contributions of plasticity toward enhanced ^{15}N uptake

Two-way ANOVA of total ^{15}N uptake revealed significant species and treatment effects but a nonsignificant interaction effect (Table 1). Multiple comparisons among treatments showed that total ^{15}N uptakes in the three nutrient levels significantly differed from each other with T_{rich} > H_{random} > T_{poor} (Fig. 3). Multiple comparisons among species showed that *S. altissima* took up significantly more total ^{15}N than the two tree species.

Pinus taeda did not demonstrate physiological plasticity in this experiment; therefore, the differences in total ^{15}N uptake among different nutrient patches were completely (100%) attributable to morphological plasticity. For *S. altissima* and *L. styraciflua*, the two species that had physiological plasticity, morphological plasticity contributed only 29% and 26%, respectively, of the enhanced ^{15}N uptake within the nutrient-enriched patches.

Mycorrhizal colonization rate

We observed lower mycorrhizal colonization in the pots than in the field for the three species. The colonization rates in pots were 0%–9.9% and 0%–9.6% for *L. styraciflua* and *S. altissima*, respectively, while in the field they were 18.0%–32.0% and 1.4%–35.7%. The colonization rates for *P. taeda* were 35.3%–61.8% in pots and 75.9%–95.0% in the field.

Discussion

Physiological plasticity in root nutrient foraging

The significant effect of the interaction between species and treatment on ^{15}N uptake rate shows that physiological plasticity differed among the three species. Within species

Fig. 2. ^{15}N uptake rate per unit fine root mass (mean \pm SE) of *Solidago altissima*, *Pinus taeda*, and *Liquidambar styraciflua* plants dosed with ^{15}N in three different nutrient levels (medium (H_{random}), high (T_{rich}), and low (T_{poor})). The three solid lines show ^{15}N uptake rates as plants were dosed with $0.58 \text{ mol}\cdot\text{L}^{-1}$ of N solution with 5 atom % ^{15}N dual labeled ammonium nitrate, while the broken line represents ^{15}N uptake per unit fine root mass of *S. altissima* dosed with $0.1 \text{ mol}\cdot\text{L}^{-1}$ of N solution with 5 atom % ^{15}N dual labeled ammonium nitrate.

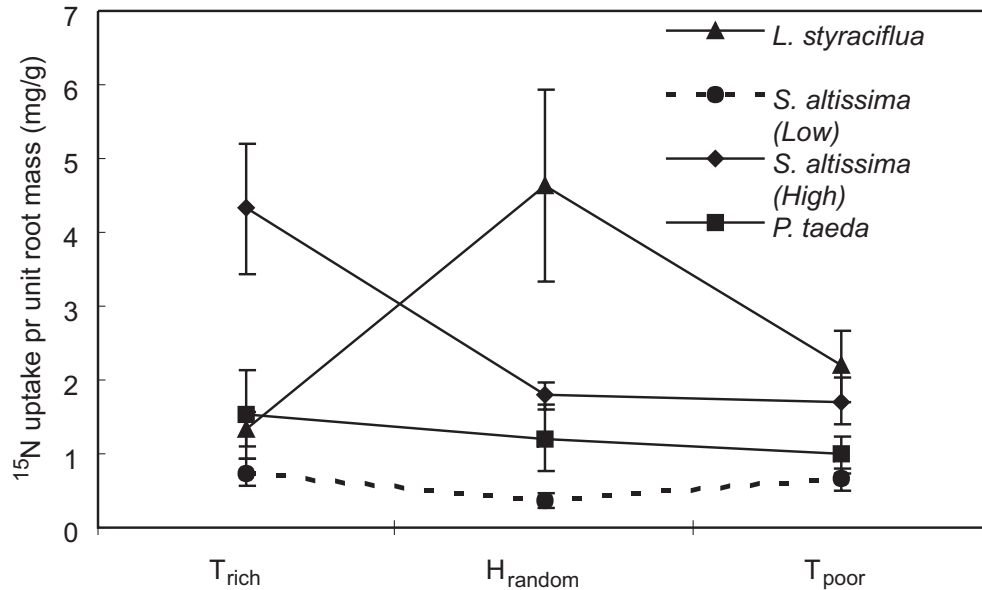


Table 3. Results of one-sample t test of relative fine root mass difference (RFRMD) values between two fertilization treatments (H vs. T) for the three species (RFRMD values indicate morphological plasticity in root nutrient foraging).

Species	RFRMD	
	H treatment	T treatment
<i>S. altissima</i>	1.006 \pm 3.945 ($n = 4$)	19.634 \pm 4.155** ($n = 7$)
<i>P. taeda</i>	5.257 \pm 2.788 ($n = 5$)	34.161 \pm 5.612*** ($n = 9$)
<i>L. styraciflua</i>	6.903 \pm 5.698 ($n = 5$)	30.475 \pm 3.580*** ($n = 7$)

Note: Values are means \pm SE (g) with sample sizes in parentheses. H_0 : RFRMD = 0. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

analyses showed nutrient uptake physiological plasticity in *S. altissima* ($p = 0.0091$) and *L. styraciflua* ($p = 0.0213$) but not in *P. taeda* ($p = 0.7770$) (Table 2). These results do not fully support our first prediction that all species show physiological plasticity.

The two species showing physiological plasticity had maximum uptake rates in different nutrient patches. *Solidago altissima*, a perennial herbaceous species, had maximum ^{15}N uptake rate in the rich patches (T_{rich}), which is consistent with previous findings (e.g., Jackson et al. 1990), while *L. styraciflua*, a deciduous tree species, had its maximum ^{15}N uptake in the medium patches (H_{random}). In a preliminary study (Bliss 2001), greater uptake rates occurred for roots growing in poor patches. Interroot competition may explain the disparity among these results. When low doses of ^{15}N are applied, it is possible that the roots will consume all of the available ^{15}N and appear to have lower uptake rates wherever root mass is higher (i.e., rich patches where roots have proliferated will appear to have lower uptake rates per unit of root mass). If, however, ^{15}N is abundant, not all of it will be consumed during a measurement period, and therefore a better comparative mea-

sure of uptake rates can be made. In our study, the comparison of the low-N dosing (two injections of $0.1 \text{ mol}\cdot\text{L}^{-1}$ N) with high-N dosing (two injections of $0.58 \text{ mol}\cdot\text{L}^{-1}$ N) in *S. altissima* provides some support for this scenario. The low dose results in the present study (Fig. 2) were similar to those in our preliminary study, which used similar concentrations of ^{15}N dosing.

Thus, although we increased the dose in this study relative to our preliminary work by more than 11 times (5.8 times the concentration per injection and two injections), interroot competition might still have affected the results for *L. styraciflua*, for which we unexpectedly observed a higher ^{15}N uptake rate in the medium than in the rich patch. There are three reasons why results for *L. styraciflua* may have been influenced by interroot competition, while results for *S. altissima* were not. First, we observed that fine roots of *S. altissima* were much more evenly distributed, while fine roots of *L. styraciflua* were more aggregated, which may have led to more intensive local interroot competition for nutrients in the latter species. Second, *S. altissima* had higher root density than *L. styraciflua* in the soil plug ($8.37 \text{ cm}/\text{cm}^3$ vs. $4.23 \text{ cm}/\text{cm}^3$), but roots of *S. altissima* were much thinner (SRL = 112) and thus may have had much narrower depletion zones than did roots of *L. styraciflua* (SRL = 78). Third, if interroot competition for nutrients did not exist, then uptake rate for *L. styraciflua* should have been at least the same in the rich as in the medium patches. However, rates were much lower in the rich patches (Fig. 2). In future studies, multiple injections of nutrient solution, but of moderate concentrations to avoid toxicity, may be needed to overwhelm effects of competition. In this study, we did not find any toxic symptom on plants or on roots in the plugs though the concentration of injected N solution was high. This suggests that performing multiple injections of a high-concentration N solution is a possible approach to examine root N uptake without acute toxicity. Our

Fig. 3. Total ^{15}N uptakes (mean \pm SE) for *Solidago altissima*, *Pinus taeda*, and *Liquidambar styraciflua* plants after they were dosed with ^{15}N in three different nutrient levels (medium (H_{random}), high (T_{rich}), and low (T_{poor})).

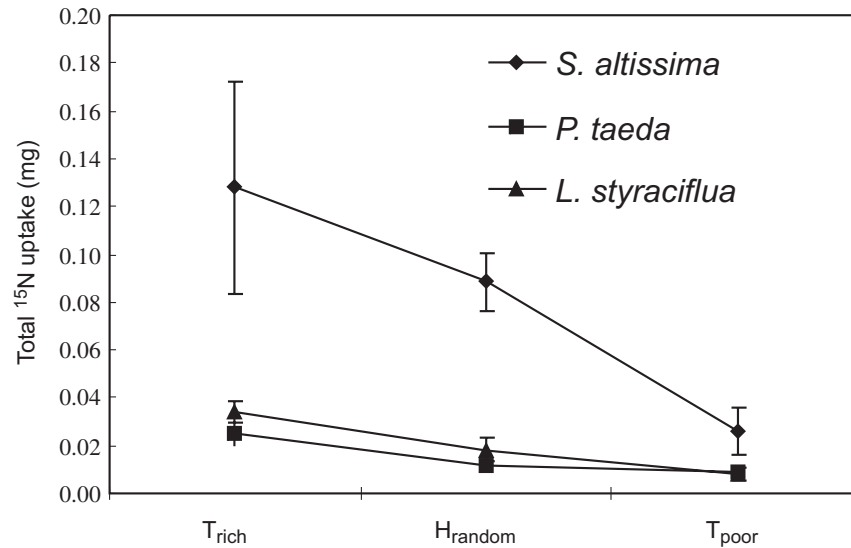
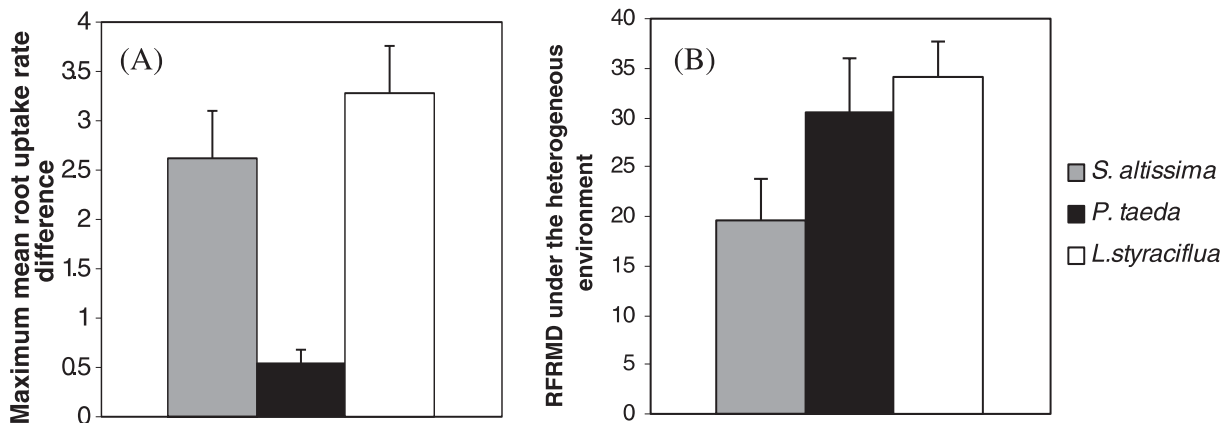


Fig. 4. Ranking of physiological plasticity and morphological plasticity of the three tested species to illustrate the relationship between the two types of root nutrient foraging plasticity. (A) Physiological plasticity (mean \pm SE) measured by maximum fine root ^{15}N uptake rate differences, and (B) morphological plasticity measured by relative fine root mass difference (RFRMD) value (mean \pm SE) for *Solidago altissima*, *Pinus taeda*, and *Liquidambar styraciflua*. In each case, greater values represent greater plasticity.



study may not have captured the maximum uptake rate and therefore the full range of plasticity in the test species. The purpose of the study was to detect plasticity, but not to estimate maximum uptake potentials, which would require an even higher ^{15}N dose than used in our study.

Contributions of the two types of plasticity to nutrient uptake increase

A surprisingly high level of enhanced N uptake was attributable to physiological plasticity. In addition, large differences among species were observed in the degree to which physiological and morphological plasticity enhanced uptake, i.e., the physiological component ranged from basically zero to as much as 74%. The results indicated not only the importance of physiological plasticity in nutrient foraging, but also that the plant species do rely on different types of nutrient foraging plasticity. The high contribution of physiological plasticity to nutrient uptake observed in two species demonstrated the effectiveness of physiological plasticity in responding to short-time release of available nutrient. Further

studies are needed to evaluate a root's lifetime contributions of physiological plasticity toward total plant nutrient uptake.

The lack of measurable physiological nutrient foraging plasticity in *P. taeda* may be a consequence of its high mycorrhizal colonization rate. Physiological plasticity variation across the three species in this study may also be related to differences in N use efficiency. In this study, leaf N concentration was significantly greater (ANOVA followed by Fisher's least significant difference multiple comparisons test $p < 0.05$), and therefore N use efficiency was lower in the two species showing physiological plasticity (1.58% in *S. altissima* and 1.43% in *L. styraciflua*) than in the species that showed no physiological plasticity (1.05% in *P. taeda*).

Relationship between physiological and morphological plasticity

This experiment has demonstrated that (1) physiological plasticity in root N uptake exists in two of the three species and differs between them; and (2) root morphological plasticity exists in the three species and differs among them.

These results provide a necessary foundation for looking at the relationship between physiological plasticity and morphological plasticity in root nutrient foraging and their contributions to nutrient uptake.

Results from three species cannot yield any general pattern on the relationship between the two types of plasticity. However, with a general expectation of a negative relationship between the two types of plasticity (Derner and Briske 1999), we expected that the order of morphological plasticity from high to low (i.e., *L. styraciflua* > *P. taeda* > *S. altissima*) should have been matched by a reverse order in physiological plasticity. *Pinus taeda* and *S. altissima* followed this expectation, but *L. styraciflua* had the highest physiological and highest morphological plasticity (Fig. 4). This suggests the possibility of “super species” that have both strong physiological and morphological plasticity in root nutrient foraging, or “inferior species” that have both weak physiological and morphological plasticity. More extensive investigation is needed to examine the relationship between the two types of nutrient-foraging plasticity.

Effect of mycorrhizal colonization on plasticity

Mycorrhizal colonizations were obviously lower in the greenhouse condition. However, our study design does not allow us to rigorously examine mycorrhizal influence on root-foraging plasticity. Cui and Caldwell (1996) found a smaller increase of root density in rich patches for mycorrhizal plants than nonmycorrhizal plants. In our previous studies on root morphological plasticity, we found that RFRMDT varied from ~20% to > 40% for *P. taeda* and from 32% to 48% for *L. styraciflua* (Mou et al. unpublished data). We suspect that variable mycorrhizal colonizations may be partially responsible for these results. Read (1992) suggested that the primary function of EM-dominated roots is to provide food base for the fungus rather than nutrient uptake. Therefore, a negative impact of EM on morphological plasticity is inevitable. However, AM may affect both types of plasticity in a less obvious manner than do EM on morphological plasticity. For example, in a recent experimental study on roots of *Plantago lanceolata* L., Hodge et al. (2000) found that an AM inoculum enhanced root proliferation but did not affect N capture from a nutrient-rich patch. Variation in colonization in plant roots may inevitably cause variation in root nutrient foraging plasticity and influence the relationship between the two types of plasticity. At this moment, however, there is no direct evidence supporting this argument.

Summary

Our results provided direct evidence that physiological plasticity in root N uptake exists in some species (*S. altissima* and *L. styraciflua*) but not in others (*P. taeda*). The data also allowed us to quantify the enhancement in N uptake due to different types of plasticity. We found that physiological plasticity contributed 71% and 74% to N uptake enhancement in *S. altissima* and *L. styraciflua*, respectively, and that morphological plasticity accounted for the remainder. In addition, the results pointed out the possibility that physiological and morphological plasticity may not necessarily be negatively correlated as suggested by other re-

searchers, and species with both high physiological and morphological plasticity may exist (*L. styraciflua* in this case). Based on the observations that (1) the highest ¹⁵N uptake rate for *L. styraciflua* occurred in H pots (medium nutrient level), and (2) N uptake did not differ among treatments when low concentrations of ¹⁵N were added to *S. altissima* pots, we believe that interroot competition may have partially masked differences in uptake rates and suggest that multiple injections with an N solution of modest concentration will be an ideal treatment condition in a future study. Finally, lower mycorrhizal colonization rates in pots than in the fields may amplify root foraging plasticity.

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Appendix A

Appendix appears on the following page.

Table A1. Procedure for calculating ^{15}N uptake rate (per unit fine root mass or length).

(1) ^{15}N concentration in each plant part ($^{15}\text{N}\%$)	$[(^{15}\text{N} \text{ atom } \% \text{ in plant part} - ^{15}\text{N} \text{ atom } \% \text{ in corresponding control plant part}) \times 15] / [(^{15}\text{N} \text{ atom } \% \text{ in plant part} - ^{15}\text{N} \text{ atom } \% \text{ in corresponding control plant part} + 1400) \times \text{N}\% \text{ in plant part}]$ (Fransen et al. 1999).
(2) ^{15}N content in each plant part (mg)	^{15}N concentration in plant part \times dry mass of corresponding plant part (fine and coarse roots inside and outside ^{15}N -treated soil plug, leaves, stems, and root stalk)
(3) Total ^{15}N uptake (mg)	^{15}N content in roots (fine and coarse) outside ^{15}N -treated soil plug + ^{15}N content in roots (fine and coarse) inside ^{15}N -treated soil plug + ^{15}N content in leaf + ^{15}N content in stem (for tree species) + ^{15}N content in root stalk (for tree species)
(4) ^{15}N uptake rate (per unit fine root mass or length) (mg/g or mg/m)	Total ^{15}N uptake / fine root mass or length in ^{15}N -treated soil plug