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CONTRASTS BETWEEN WHOLE-PLANT AND LOCAL NUTRIENT LEVELS DETERMINE ROOT GROWTH AND DEATH IN *AILANTHUS ALTISSIMA* (SIMAROUBACEAE)[1]

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- *Premise of the study:* There is an ongoing debate about the importance of whole-plant control vs. local modular mechanisms for root growth. We conducted a split-root experiment with different patch/background levels of nitrogen to examine whether local root growth and death are controlled by local resource levels or at the whole-plant level.
- *Methods:* Three microrhizotrons with 0, 10, and 100 μg N/g growth medium levels (74 g growth medium each) were attached to pots of high or low soil N in which one *Ailanthus altissima* individual was growing. One fine root was guided into each of the microrhizotrons and photographed every 4 d. Plants were harvested after 28 d; root growth and mortality in the microrhizotrons were recorded. Changes in root length, number of laterals, and interlateral length were determined from the photos and analyzed.
- *Key results:* While overall plant growth was influenced by background N level, both patch and background N levels influenced root growth and mortality in patches. Local roots proliferated most when the patch N level was high and background level low, and they proliferated least and showed highest mortality when patch N was low and the background level high.
- *Conclusions:* The fate of roots growing in a patch is influenced by the resource environment of the plant's other roots as well as the resource levels in the patch itself. Thus, the growth and death of roots in patches is determined by both modular and whole-plant mechanisms.

Key words: *Ailanthus altissima*; nitrogen levels; root modularity; root plasticity; Simaroubaceae; soil heterogeneity; soil patches; tree of heaven.

Soil resources are distributed heterogeneously (Jackson and Caldwell, 1993; Stewart et al., 2000). Root plastic responses to environmental heterogeneity may be critical for effective nutrient uptake by plants (Hodge, 2004, 2006). Plant roots are able to show several forms of phenotypic plasticity in response to spatial and temporal resource variability (Drew and Saker, 1975; Gersani and Sachs, 1992; Gross et al., 1993; Mou et al., 1997; Wang et al., 2006). Plant growth aboveground is modular (Harper, 1977), and modules are often semiautonomous (Preston and Ackerly, 2004). There is evidence that plant roots can also behave as modules that respond independently to their own local resource environment (de Kroon et al., 2005). There has been an ongoing debate about the degree of module autonomy (Watson, 1986) vs. whole-plant integration (Novoplansky et al., 1989) of aboveground organs. There is now a similar debate with respect to root plasticity.

Based upon modularity and cost-benefit theory, Eissenstat and Yanai (1997) proposed the concept of root efficiency, defined as nutrient uptake per unit C cost (root construction + maintenance per unit of root length or mass). Following this line of reasoning, and assuming that the C cost per unit root length (or mass or surface area) is relatively constant, we hypothesized that growth or death of a root segment is determined by the local resource level with a response time lag, and we recently tested this hypothesis in a split-root experiment (Hu and Mou, 2013). Consistent with previous work, the results supported the prediction that root growth was promoted by higher local N level. But root death was not observed in low nutrient patches, even when the N levels in the patches were extremely low.

The responses of a root to a resource patch may depend not only on the nutrient availability in the patch but also on the contrasts among the nutrient contents in other patches or overall (Friend et al., 1990; Wijesinghe and Hutchings, 1999; Blouin and Puga-Freitas, 2011). Most studies examining root plasticity have focused on the responses to the level of resource in patches (Campbell and Grime, 1989; Einsmann et al., 1999; Wang et al., 2006), but little attention has been paid to the possible effects of contrasts among nutrient levels experienced by different roots of the plant (Kotliar and Wiens, 1990; Lamb et al., 2004). For example, a plant root may respond to a low resource patch differently if other roots from the same plant are in higher resource environments than if the other roots are also experiencing low resource levels, as has been shown for shoots (Snow, 1931; Novoplansky et al., 1989). A few modeling studies have considered the effects of contrasts among patch nutrient levels. Some modeling studies predict that organisms will allocate more efforts to patches where local resource levels are high or when the overall resource level is low (McNickle et al., 2009). Other modeling studies predict that plants will place more roots into

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nutrient-rich patches when contrasts among the patches are higher (Fransen et al., 1999).

There are few reports of controlled experiments on this question. Root proliferation in *Glechoma hederacea* appears to be highly sensitive to the degree of contrast between patches (Wijesinghe and Hutchings, 1999). The proportion of root biomass in enriched patches was significantly altered by the difference with other patches, and root to shoot ratios rose as the patch contrast increased, even though the overall resource level was unchanged. There was a significant effect of the contrast between poor and rich patches on the lateral root density and root elongation in *Arabidopsis thaliana* (Blouin and Puga-Freitas, 2011). On the other hand, root mass, shoot mass, and C allocation to roots of *Abutilon theophrasti* did not respond to the degree of contrast in resource levels between rich patches and the background, but these contrasts may have been too weak to be detected by the plants (Lamb et al., 2004). In addition to the effects of patchy resources, whole-plant internal N status can exert control on lateral root development (de Kroon et al., 2009). When *Arabidopsis thaliana* was growing under N-deficient conditions, lateral root growth was increased, while a high tissue N concentration suppressed lateral root growth (Ruffel et al., 2011).

Resource availability has major effects on fine-root mortality (Schoettle and Fahey, 1994), and soil N may be the primary determinant (Block et al., 2006). Our lack of information concerning the mechanisms controlling the death of individual roots has been labeled "one of the most remarkable gaps in our knowledge" (Pregitzer, 2002). Several studies have demonstrated that the mortality of fine-root is a response to nutrient availability, but the results have not been consistent concerning whether fertile soils reduce or promote root mortality (Pregitzer et al., 1993; Burton et al., 2000; King et al., 2002; Adams et al., 2013). The supply and duration of soil resources can influence root mortality, while addition of water and nitrogen can extend the lifespan of roots (Pregitzer et al., 1993). Average root lifespan in a northern hardwood forest dominated by *Acer saccharum* was longer when N availability was greater (Burton et al., 2000). Fine-root mortality rates of *Pinus taeda* were significantly higher in fertilized than in the control treatment (King et al., 2002). Root mortality of *Picea abies* increased significantly in fertilized compared with control plots (Majdi, 2001), but nutrient heterogeneity in these studies was at a "coarse-grained" scale, i.e., larger than the root system of an individual plant, so there was no information about the behavior of different roots on the same plant. The scale of soil resource heterogeneity in the field is often finer than this.

Higher patch resource availability stimulates root proliferation (defined as initiation of new lateral roots, Hodge, 2004) in almost all species that have been studied (Hodge, 2009). For whole root systems or at the stand level, higher soil nutrients, usually N, often lead to significantly lower root to shoot ratios indicating decreased root productivity (Bonifas et al., 2005), although this can be a size (i.e., allometric) effect, as the root to shoot ratio decreases with size as plants grow (Müller et al., 2000). The different responses to the soil nutrient levels at different spatial scales lead to the hypothesis that both responses to local heterogeneity and whole-plant integration interact to regulate the root growth (Robert and Friml, 2009).

Applying optimality theory (Bloom et al., 1985; Eissenstat and Yanai, 1997; Davis et al., 2000) to our current knowledge of the relationships between root growth/plasticity and soil N level at different scales of heterogeneity, leads to the following predictions: (1) Positive nutrient patches (i.e., patch nutrient level > background nutrient level) promote root growth via elongation and new root proliferation. (2) Negative patches (patch nutrient level < background nutrient level) promote root senescence and death. (3) Increased contrast between background and patch results in increased differences in growth or senescence.

In the present study, we used a split-root experiment to give three root segments from individual plants different nitrogen (N) levels with both high and low background levels (i.e., where most of the plant's root system were growing). This modified split-root treatment allowed us to examine the growth and mortality of fine roots and test the above hypotheses. To ensure the data reflect absorbing, fine roots, we confined our study to 1st and 2nd order primary or lateral roots.

MATERIALS AND METHODS

***Experimental design*—**A split-root experiment was conducted in an environmentally controlled greenhouse at Beijing Normal University in 2012. *Ailanthus altissima* (Mill.) Swingle (tree of heaven; Simaroubaceae), a native tree species in a vast area of the eastern Asian temperate region and an invasive in North America and some other parts of the world, was chosen as the experimental species. The species was chosen because in our previous experiments it showed a high degree of morphological plasticity in response to nutrient patches. Seeds were purchased from the Agricultural Academy of Beijing.

The seeds were sowed in germination pans containing moist sand 4-cm deep with 1 cm of sand on top of the seeds. Seedlings were transplanted into 8-L plastic pots (top diameter 28 cm, bottom diameter 24 cm, height 15 cm) when they had 4–5 true leaves (about 30 d after sowing). The pots were filled with 7 L of fine construction sand with very low nutrient content, then irrigated with 40 mL of 7.14 mmol/L N nutrient solution (3.94% NH_4-N, 6.05% NO_3-N, 10.01% urea-N, 20% P_2O_5 20% K_2O, Peters Professional, Scotts, Marysville, Ohio, USA) when seedlings were transplanted, and once per week until all were established.

The spilt-root treatment was initiated 120 d after transplanting, when the plants were large enough to have extended their roots to the edge of the pots (determined by harvesting extra planted pots biweekly). By carefully brushing out the sand to expose the top branches of the root system, three healthy 1st order root segments (~2 cm long) were inserted into three different microrhizotrons (mrhizo hereafter) built for this purpose (Fig. 1). Each mrhizo was connected to the pot by inserting it into one of the three 10×1 cm slots cuts into the pot, then fixed. The mrhizo was made of two pieces of 13.8×8.8 cm transparent plastic (polymethyl methacrylate) glued parallel to one another with 0.4×0.5 cm black plastic sidebars in between. The ~54 cm^3 mrhizo space was filled with the 40-mesh white quartz sand, and the root was inserted close to the lower glass, with a piece of 200 mesh nylon net between the root and the sand to prevent the root from growing into the sand to facilitate photography. Twelve holes of 2 mm diameter were drilled in the upper glass of each mrhizo and evenly distributed on the glass for irrigation and fertilization, and a 2-cm grid system was drawn on the lower glass of the mrhizo as a reference for photos. The mrhizos were fixed to the pots with metal wires. Two opaque plastic plates colored black inside and white on the outside were attached outside the mrhizos to prevent rapid light and temperature fluctuations (Fig. 1).

The greenhouse where the plants grew was set to 16-h light/8-h dark. The air temperature ranged between 23°C and 28°C during the day and between 15°C and 20°C at night. The relative humidity was maintained at 35 ± 5% during the day and 55 ± 5% at night. Light intensity at the top of the plant canopy was approximately 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetic photon flux density (PPFD) during the day.

***Treatments and root photography*—**The treatments were applied at the time of the root splitting procedure. Deionized water or modified Hoagland solution was injected to the mrhizos to create three N levels (0 μg N/g [low mrhizo hereafter], 10 μg N/g [medium mrhizo], and 100 μg N/g [high mrhizo]) for each of the experimental plants. We injected 0.6 mL deionized water per hole (7.2 mL in all per mrhizo) into the mrhizos for the low mrhizo treatment, 0.6 ml half-strength Hoagland solution (7.5 mmol/L N) per hole for the medium mrhizo, and 5× Hoagland solution (75 mmol/L N) for the high mrhizo treatment. The modified Hoagland solution contained 4 mmol/L $Ca(NO_3)_2 \cdot 4H_2O$,

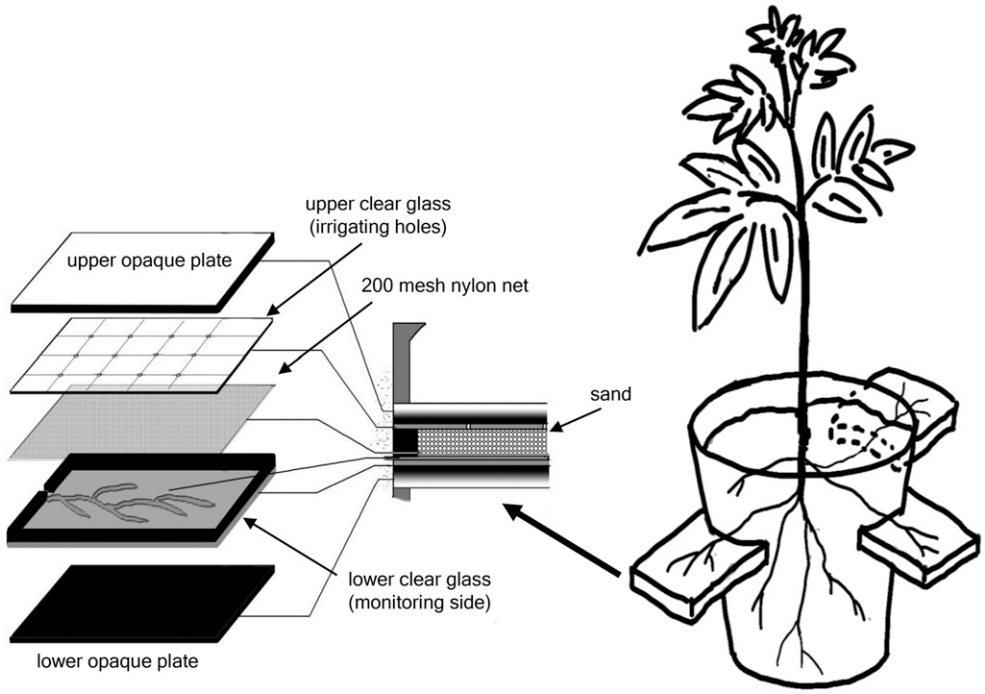

Fig. 1. Design of the mrhizo apparatus and an illustration of the split-root experiment. The three mrhizos attached to the pot were randomly assigned 0, 10, and 100 μg N/g treatments, and the pot (background) was assigned either high or low N treatment.

5 mmol/L KNO_3, 1 mmol/L NH_4NO_3, 1 mmol/L KH_2PO_4, 2 mmol/L $MgSO_4 \cdot 7H_2O$, 50 mmol/L $Fe^{2+}Na_2EDTA$ and micronutrients. The three N levels were randomly assigned to the three mrhizos on each pot. Pots were randomly assigned to high or low N background.

The low N background pots were watered with 60 mL 1/2-strength Hoagland solution, and the high N background pots received 120 mL 5× Hoagland solution every 8 d. The two background levels and three N-level patch treatments provided six levels of positive and negative contrasts. There were a total 12 pots in each of the high N background and low N background groups. Thus, the whole experiment is a 2×3 complete randomized design with 12 replications. To leach the sand and avoid build up of mineral salts, the pots were watered excessively before the second, third, and the fourth nutrient applications. The pots were watered with mist (about 200–300 mL per pot) when the soil surface was dry (usually 4 d after each nutrient application) to avoid water stress, and extra holes were punched in the bottom of the pots to avoid waterlogging. The pots were randomly rearranged every 4 d. Fungicide (thiophanate-methyl) and insecticide (avermectin-acetamiprid) were sprayed biweekly throughout the experiment.

Fifteen additional plant-free pots with sand and mrhizos were set up to monitor the variation of available N in the mrhizos and the pots. N levels of the high N background pots were significantly higher than that of the low N background pots. No N exchange between the pots and the mrhizos was detected. Available N in the mrhizos was relatively constant over time and significantly different among the three N levels throughout the experiment (Appendix S1, see Supplemental Data with the online version of this article).

The root growth in the mrhizos was monitored every 4 d by photographing roots under the lower mrhizo glass from a distance of 15 cm with a digital camera (Sony DSC-H50). Each mrhizo was then watered with deionized water through the 12 holes. In the low N background pots, two roots showed insect bite marks, one in a low mrhizo, and one in a high mrhizo. These were excluded from the analysis. WinRHIZO Tron 2009a software (Regent Instruments, Quebec City, Quebec, Canada) was used to analyze the root growth from the photos. The root growth of the patches was determined by growth in root length (linear elongation), number of laterals, and interbranch length of the 1st order laterals.

***Plant harvest*—**The mrhizo roots were harvested 29 d after the first treatment application when most of the high mrhizo/low N background had roots that extended to the mrhizo edges (Appendix S2, A; see Supplemental Data with the online version of this article). More than 95% of the mrhizo roots were 1st or 2nd order, fine, absorbing roots. Root mortality was evaluated when the mrhizo was opened. A root was considered dead if it was shrunken, hollow, or stuck to the glass. All the roots that died during the 28-d period showed little previous growth (Appendix S2, C). No live roots had dead root segments. Whole plants were then harvested and separated into fine roots, coarse roots, taproot, stem, and leaves. Fine roots were of 1st and 2nd orders plus any non-suberized (by visual observation under magnified lens) 3rd order roots. Coarse roots were of the suberized 3rd and higher orders, and the taproot was directly connected to the stem. All the plant parts were oven-dried to constant mass at 65°C and weighed. They were then ground, passed through an 80-mesh sieve and N concentration measured using organic elemental analyzer Vario EL cube (Elementar Analysensysteme GmbH, Hanau, Germany) in the laboratory of the Beijing Chemical Engineering University.

***Data analysis*—**Root length, number of laterals, and interbranch length of 1st order laterals were log-transformed where necessary to improve normality and homoscedasticity. Because the factor mrhizo was nested within background, the root length, number of laterals and interbranch length of 1st order laterals were analyzed using a nested two-way, repeated-measures ANOVA (Proc GLM in SAS, SAS Institute, Cary, North Carolina, USA). In cases where the assumption of data sphericity was violated, the results of the within-subjects analyses were corrected using the Greenhouse–Geisser method (von Ende, 2001). To compare the differences among the mrhizos, the data were divided into two groups: high vs. low background N levels. Repeated measures ANOVA was carried out separately in each group for the three growth parameters. If the effect of mrhizo was significant, Duncan's multiple comparison was used for posthoc analysis. Because mortality is a binary variable (living vs. dead), logistic regression was used to analyze the influence of mrhizo/background N contrast on the probability of root death.

The results of the repeated measures ANOVA and logistic regression modeling together were used to examine the predictions on the effects of positive/negative patch contrasts on the growth and death of the mrhizo roots. If the three measured parameters in the high positive patches (such as high mrhizo/low N background) are significantly higher than the others and mortality was lower, our first prediction would be supported. If roots in the high negative patches (such as low mrhizo/high N background) show the opposite results (i.e., the three parameters are lower than the others, while deaths were higher), the second prediction would be supported. The third prediction was examined by comparing the data from the most positive (high mrhizo/low N background)

and the most negative (low mrhizo/high N background) contrasts with the moderately positive and negative contrasts.

The final biomass, N concentration, and C to N ratios of the leaves, stem, taproot, coarse roots, and fine roots of the plants under high and low N background levels were analyzed with a two-way MANOVA (protected ANOVA approach, Scheiner, 2001) due to possible correlations among the parameters (biomass, N concentration, and C to N ratio). If the N background × plant parts interaction was significant, Duncan's multiple comparison was used to compare differences among plant parts as a posthoc analysis. This analysis examines the growth differences affected by the two background N treatments. In addition, root to shoot ratio, shoot to fine-root ratio, leaf to fine-root ratio of each plant, and their statistics were computed. All statistical analyses other than nested repeated measures ANOVA were performed by SPSS v. 20 software (IBM, Chicago, Illinois, USA).

RESULTS

Root growth in the mrhizo treatments—The results of the between-subject effect in the nested repeated measures ANOVA showed that the root length, the number of laterals, and the interbranch length of the 1st order laterals were all significantly influenced by mrhizo N level and background N level (Table 1). There were significant differences among the repeated measures of the three mrhizos of a plant, and the root growth parameters responded to mrhizo N levels differently in high N background vs. low N background conditions. Under the high N background condition, root length and number of laterals were not significantly different between the low mrhizo and medium mrhizo during the whole experiment, but both were significantly lower ($F_{2, 33} = 3.646$, $P < 0.05$ for root length, $F_{2, 33} = 3.831$, $P < 0.05$ for number of laterals) than that of the high mrhizo (Fig. 2B, D). The root length and the number of laterals did not increase with time in the low mrhizo and medium mrhizo, while those in the high mrhizo increased significantly (Fig. 2B, D). Under the low N background condition, root length and number of laterals of high mrhizo were significantly greater ($F_{2, 31} = 22.161$, $P < 0.001$ for root length, $F_{2, 31} = 29.704$, $P < 0.001$ for number of laterals) than those of the low mrhizo and medium mrhizo. The root length was not significantly different between the low mrhizo and medium mrhizo throughout the entire experiment, but the numbers of laterals differed significantly between the low mrhizo and medium mrhizo ($F_{2, 31} = 29.704$, $P < 0.001$, Fig. 2A, C). Neither the root length nor the number of laterals in the low mrhizo increased over time, but they increased somewhat in the medium mrhizo and increased continuously over the course of the experiment in the high mrhizo (Fig. 2A, C).

Under the high N background N level, the interbranch lengths of the 1st order laterals showed no significant difference in the low mrhizo vs. the medium mrhizo over the course of the experiment. The interbranch length of the high mrhizo was significantly ($F_{2, 33} = 4.523$, $P < 0.05$) lower than that in medium and low mrhizo treatment (Fig. 2F). Under the low N background condition, the interbranch length showed significant differences ($F_{2, 31} = 22.573$, $P < 0.001$) among all three mrhizo N levels. Interbranch length was relatively stable over time in the low mrhizo, decreased slightly in the medium mrhizo, and decreased greatly in the high mrhizo (Fig. 2E).

Root mortality under different patch/background N contrasts—In a two-variate (background and mrhizo N level) logistic regression analysis on the probability of a mrhizo root dying, both background ($P < 0.001$, Table 2) and mrhizo N levels ($P < 0.05$, Table 2) significantly predicted the probability of root death in patches. Using the fit equation, the probabilities of root death 28 d after the treatments were estimated to be 0.363, 0.283, and 0.056 for low mrhizo, medium mrhizo, and high mrhizo, respectively, in the low N background, and 0.917, 0.884, and 0.532 in the high N background (Table 3).

We monitored available N concentration in background pots and mrhizos (Appendix S1). We defined mrhizo/background N contrast as the available N concentration in mrhizo divided by that in background pot soil. Soil mrhizo/background N contrasts are negative if they are less than 1, and positive if they are greater than 1. In the sequential monitoring data of available soil N, the mean contrasts were calculated as 0.04 (±0.007), 0.23 (±0.022), and 2.19 (±0.249), respectively, for low mrhizo, medium mrhizo, and high mrhizo in the high N background pots (average 35.8 μg N/g) and as 0.28 (±0.026), 1.80 (±0.124), and 16.19 (±0.829) in the low N background pots (average 5.03 μg N/g) (Table 3).

Biomass, N concentration, and C to N ratio of different plant parts—The effects of background, plant parts, and their interaction were all significant (MANOVA, Pillai's trace < 0.001, Table 4). Subsequent protected ANOVAs showed that the biomass, N concentration, and C to N ratio of different plant parts differed significantly ($P < 0.001$, Table 4), the background nutrient treatments significantly influenced them ($P < 0.001$, Table 4), and there was a significant interaction between the background nutrient treatments and plant parts ($P < 0.01$ for biomass, $P < 0.001$ for N concentration and C to N ratio, Table 4).

TABLE 1. Results of nested repeated measures ANOVA for root length, number of lateral roots, and interbranch length of 1st order lateral roots of *Ailanthus altissima* grown in the 0, 10, and 100 μg N/g mrhizos under the high and low background N levels.

	Root length			Number of laterals			Interbranch length of 1st order laterals		
Source	df	MS	*F*	df	MS	*F*	df	MS	*F*
Between-subject effects									
Background	1	9.93	21.69***	1	16.36	24.87***	1	4.39	28.63***
Mrhizo (Background)	4	6.17	13.48***	4	11.06	16.82***	4	2.23	14.54***
Error	64	0.46		64	0.66		64	0.15	
Within-subject effects									
Time	1.24	5.48	59.78***	1.34	7.68	62.86***	2.09	0.83	49.18***
Time × Background	1.24	1.98	21.61***	1.34	2.67	21.85***	2.09	0.31	18.40***
Time × Mrhizo (Background)	4.97	1.63	17.75***	5.37	2.07	16.94***	8.32	0.16	9.69***
Error	79.51	0.09		85.88	0.12		133.64	0.02	

Notes: Sphericity of the data could not be achieved, so df and MS was corrected with Greenhouse–Geisser's ε. *** $P < 0.001$.

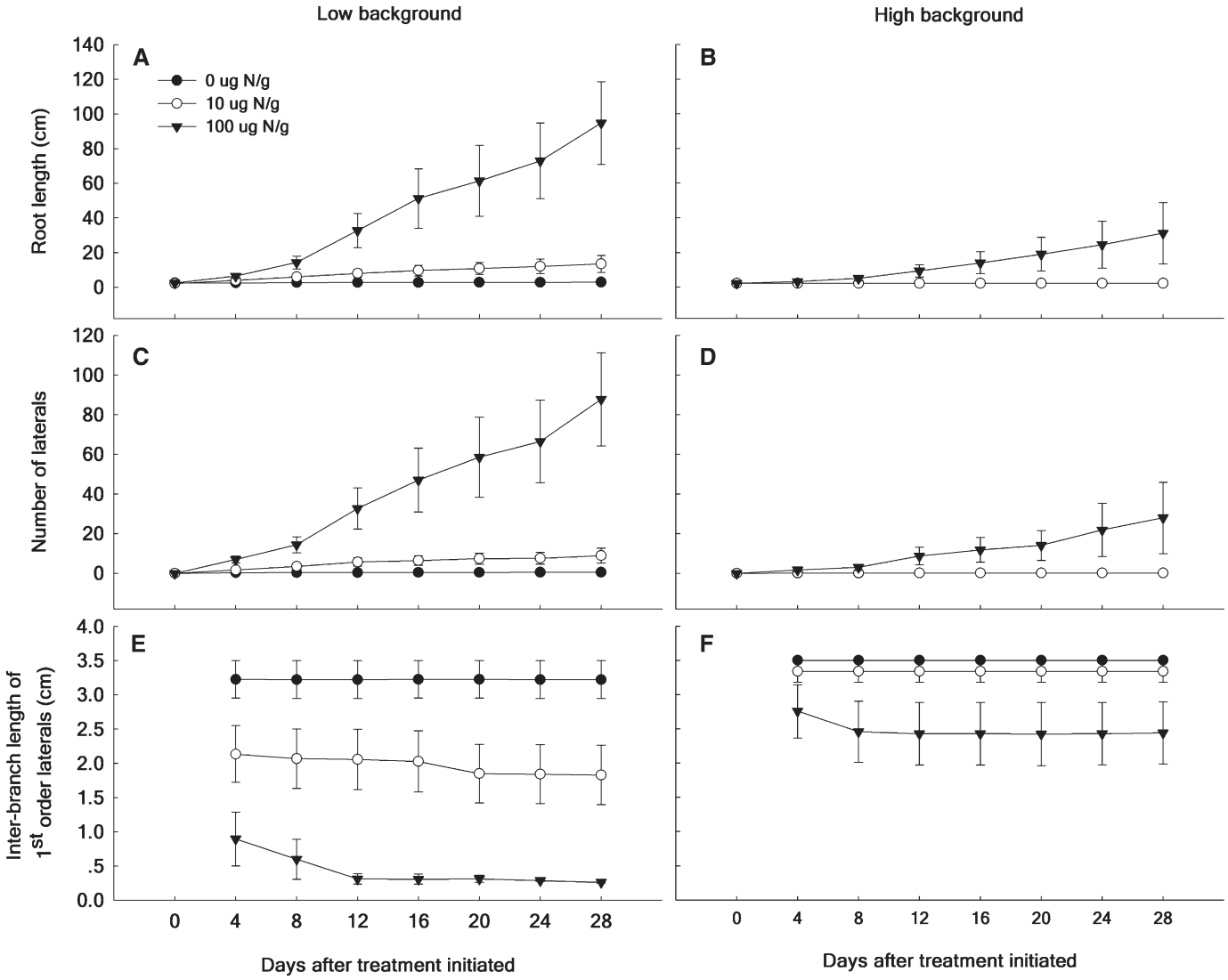

Fig. 2. Temporal changes in (A, B) root length, (C, D) number of laterals, and (E, F) interbranch length of the 1st order lateral roots of *Ailanthus altissima* in the 0, 10, and 100 μg N/g mrhizos under the low (A, C, E) and high (B, D, F) background N levels. Error bars indicate SE, $n = 11$ for 0 and 100 μg N/g mrhizos in low background pots, $n = 12$ for others.

The biomass and N concentration of different parts were significantly higher in high N background than in low N background, while the C to N ratios were significantly higher in low N background (Fig. 3). The biomass and N concentration of leaves in the high N background pots were much higher than that of the other parts, but this trend was much smaller in the low N background pots (Fig. 3A, B). Taproots had the lowest N concentration and therefore the highest C to N ratio. High N background pots had higher N concentration and lower C to N ratios for all plant parts (Fig. 3).

TABLE 2. Parameter estimates from logistic regression and their statistics for prediction of root death in mrhizos at three levels of N, and high or low background N level. The logistic regression model is $P(D) = \exp(a + b\text{BG} + c\text{PA} + d\text{PB})/[1 + \exp(a + b\text{BG} + c\text{PA} + d\text{PB})]$, where $P(D)$ is the death probability of root in a patch, BG is the background N concentration (low N background = 0, high N background = 1), PA and PB (each acquiring value of 1 or 0) used together to denote the 3 mrhizo N levels, i.e., PA = 1 and PB = 0 for low mrhizo, PA = 0 and PB = 1 for medium mrhizo, and both equal 0 for high mrhizo.

Variable	B	SE	Wald	df	*P*
BG (*b*)	2.962	0.712	17.319	1	<0.000***
PA (*c*)	2.272	0.858	7.008	1	0.008**
PB (*d*)	1.901	0.825	5.306	1	0.021*
Constant (*a*)	−2.833	0.798	12.609	1	<0.000 ***

DISCUSSION

Background/patch contrasts, root growth, and root mortality— The roots of *A. altissima* in positive patches (i.e., patches in which the nutrient levels were higher than the background including combinations of medium mrhizo /low N background, high mrhizo/low N background, and high mrhizo/high N background), showed more growth in root length and proliferation than roots in the negative patches, where roots showed little growth and high mortality rates. These results are consistent with our first and second predictions. Furthermore, roots in the

TABLE 3. Background/mrhizo contrast and root death probability in mrhizos at three N levels, and high vs. low background N levels.

Background N level	Mrhizo N level (μg N/g)	Background/ mrhizo contrast	Root death probability in mrhizo
H	100	2.19 (±0.249)	0.532
	10	0.23 (±0.0215)	0.884
	0	0.04 (±0.0072)	0.917
L	100	16.19 (±0.829)	0.056
	10	1.80 (±0.124)	0.283
	0	0.28 (±0.026)	0.363

most positive patches (i.e., high mrhizo/low N background where [mrhizo N]/[background N] = 16.19) revealed the highest root growth in all measures (Fig. 2), while the most negative patches (i.e., low mrhizo/high N background where [mrhizo N]/ [background N] = 0.04) had the highest probability of root mortality (91.7%; Table 3), supporting our third prediction.

Some discrepancies from our predictions emerged when the information on root growth (Fig. 2) and the probability of root mortality were examined. The three root growth parameters (Fig. 2) and the probability of mortality (Table 3) in the high mrhizo patches showed large differences between the low (high mrhizo/low background N = 16.19) and the high N backgrounds (high mrhizo/high background N = 2.19, Table 3). These results demonstrate that roots may respond differently to the same N availability, depending on the nutrient conditions of the remaining root system, which means the mrhizo/background N contrasts may affect patch root growth and mortality. But even similar mrhizo/background N contrasts elicited different root responses. The high mrhizo/high N background (2.19) and medium mrhizo/low N background (1.80) had similar positive contrasts, but showed different root growth and mortality, with higher root growth (Fig. 2), and higher mortality (Table 3) in the high mrhizo/high N background. Similarly, low mrhizo/low N background and medium mrhizo/high N background had similar negative contrasts (0.28 vs. 0.23 respectively), both showed little root growth, but the mortality rates were very different (36.3% vs. 88.4%, respectively, Table 3). These results indicate that whole-plant conditions, determined by the background N treatment, had an overriding influence on root growth and mortality. This corresponds to what has been observed for shoots on the same plant in high and low light environments (Snow, 1931; Novoplansky et al., 1989). When one of two branches on a plant was in the sun, branches in the shade were sacrificed, presumably because their cost was greater than benefit. When both branches were in the shade, they were kept alive as long as possible. Similarly, in our experiment, roots with a low N background endured low patch nutrient availability much longer than those in the high N background.

Several researchers have noted that contrasts in resource levels are an important aspect of heterogeneity (Kotliar and Wiens, 1990; Lamb et al., 2004) and that the overall nutrient content of different contrasts can vary, confounding the influences of differences in soil nutrient levels on plant biomass growth (Lamb et al., 2004; Blouin and Puga-Freitas, 2011). Lateral root proliferation and elongation of barley (*Hordeum vulgare* cv. Proctor) in N-rich patches were promoted only when N in the surrounding patched was low (Drew and Saker, 1975). A study on *Glechoma hederacea* by Wijesinghe and Hutchings (1999) revealed the influence of patch contrast when good and poor patches were relatively large and equal in size and number. However, few have reported local root mortality as a result of nutrient contrasts. Our experimental patches were fine-grained relative to the plants, and we emphasize the role of a background (large patch) and local patch resource level on growth and death of roots in the local patches. Our data suggest that the local response of roots to patch nutrient availability is not determined primarily by contrasts among patches per se, but by the overall nutrient conditions experienced by the plant.

Local responses and plant level responses—As expected, the biomass, N concentration, and C to N ratios of the experimental plant parts, as well as whole plants, were consistently different between high N background and low N background conditions (Fig. 3). The root to shoot and fine root to leaf ratios of the plants in the low N background pots were twice as high as those of the plants in the high N background pots. The results changed little when the mass of the high mrhizo roots were added, indicating that the influence of the patches at the whole-plant level was not significant, at least over the short period of the experiment.

In contrast to the small effects at the whole-plant level, both background and patch nutrient levels influenced growth and death of mrhizo roots (Table 1) as discussed previously. The different local- vs. plant-level responses of root growth and death further indicated that small, extreme patch/background contrasts amplify the aforementioned root growth and death trends. These further support our predictions from a slightly different perspective.

When did plant roots die?—Not all the dead mrhizo roots died near the end of the experiment. Few roots in the low mrhizo patches showed length growth and new initiation of the laterals before death (Fig. 2), but they had much higher death rates (91.7%) under high N background than that (36.3%) under low N background. The data from other mrhizo pairs showed

TABLE 4. Results of MANOVA and protected ANOVA for the biomass, N concentration, and the C to N ratio (C/N) of the different parts of the individual plants of *Ailanthus altissima* under the high and low background N levels.

	Multivariate tests			Protected ANOVA results					
				Biomass		N concentration		C/N ratio	
Source	Hypothesis df	Error df	*F*	df	*F*	df	*F*	df	*F*
Background	3	108	242.65***	1	77.93***	1	415.91***	1	511.46***
Parts	12	330	36.94***	4	26.08***	4	435.58***	4	482.78***
Background × Parts	12	330	13.80***	4	5.35**	4	16.13***	4	10.53***
Error				110		110		110	

Notes: Pillai's trace is used for the results of multivariate tests. ** $P < 0.01$, *** $P < 0.001$.

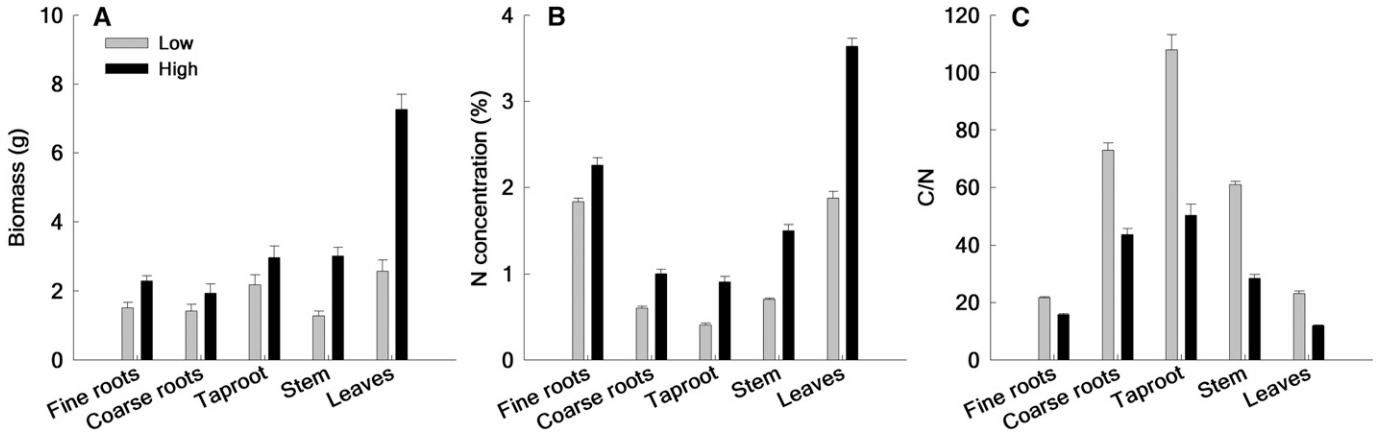

Fig. 3. Comparisons of (A) biomass, (B) N concentration, and (C) C to N ratio (C/N) of plant parts of experimental *Ailanthus altissima* individuals grown under the high and low background N levels. Error bars indicate SE, $n = 12$.

similar contrasts in root death probabilities to those described above. These indicate that the roots in the high N background die sooner than those in the low N background. It was not possible to observe root mortality over time through sequential observation of the mrhizo because the dead fine roots of *A. altissima* are very difficult to distinguish visually. Advances in technology may allow us to do this in the future.

Modularity and plant level regulation—Harper (1977) viewed a plant as a population of modules and emphasized the independence of these modules in term of initiation, development, and death. His views influenced ecologists to consider phenotypic plasticity of plant modules as a local rather than whole-plant response. As summarized by de Kroon et al. (2005, p. 73), "...whole-plant reaction norms are an integrative by-product of modular plasticity." Studies on genetic and physiological mechanisms of root development, mostly in *Arabidopsis thaliana*, revealed the importance of plant control through complex hormonal interactions responding to local nitrate signals (Zhang and Forde, 1998; Malamy, 2005; Zhang et al., 2007; Ruffel et al., 2011) supporting the idea that root modular development results from local responses interacting with some systemic control (de Kroon et al., 2009).

The significant differences in the growth parameters of the three roots, similar to other split-root experiments, as well as the different death probabilities from different mrhizo N levels, demonstrate the modularity of root plasticity in responding to local resource levels. Significant background N levels on growth and death of roots are evidence for integrated, systemic control of local root growth and death by the plant when confronted with spatial contrasts in nutrient levels. Such integration is presumably hormone-mediated (Aloni et al., 2006; Robert and Friml, 2009).

In summary, soil mrhizo/background N contrasts promoted root growth in positive patches, while negative contrasts promoted root death. In addition to its contribution to patch/background contrasts, the background N level influenced the overall plant performance. Our results also suggest that the size of a patch relative to the background may also influence the growth and death of the root modules growing there: the smaller the patch size (higher spatial contrast), and the higher the resource contrast, the stronger the local modular response will be. More experiments should be conducted to examine the variation of both root morphological plasticity and the underlying physiological mechanisms to better understand the interplay of local responses and whole-plant control.

LITERATURE CITED

ADAMS, T. S., M. L. MCCORMACK, AND D. M. EISSENSTAT. 2013. Foraging strategies in trees of different root morphology: The role of root lifespan. *Tree Physiology* 33: 940–948.

ALONI, R., E. ALONI, M. LANGHANS, AND C. I. ULLRICH. 2006. Role of cytokinin and auxin in shaping root architecture: Regulating vascular differentiation, lateral root initiation, root apical dominance and root gravitropism. *Annals of Botany* 97: 883–893.

BLOCK, R. M. A., K. C. J. VAN REES, AND J. D. KNIGHT. 2006. A review of fine root dynamics in *Populus* plantations. *Agroforestry Systems* 67: 73–84.

BLOOM, A. J., F. S. CHAPIN III, AND H. A. MOONEY. 1985. Resource limitation in plants—An economic analogy. *Annual Review of Ecology and Systematics* 16: 362–392.

BLOUIN, M., AND R. PUGA-FREITAS. 2011. Combined effects of contrast between poor and rich patches and overall nitrate concentration on *Arabidopsis thaliana* root system structure. *Functional Plant Biology* 38: 364–371.

BONIFAS, K. D., D. T. WALTERS, AND K. G. CASSMAN. 2005. Nitrogen supply affects root:shoot ratio in corn and velvetleaf (*Abutilon theophrasti*). *Weed Science* 53: 670–675.

BURTON, A. J., K. S. PREGITZER, AND R. L. HENDRICK. 2000. Relationships between fine root dynamics and nitrogen availability in Michigan northern hardwood forests. *Oecologia* 125: 389–399.

CAMPBELL, B. D., AND J. P. GRIME. 1989. A new method of exposing developing root systems to controlled patchiness in mineral nutrient supply. *Annals of Botany* 63: 395–400.

DAVIS, M. A., J. P. GRIME, AND K. THOMPSON. 2000. Fluctuating resources in plant communities: A general theory of invisibility. *Journal of Ecology* 88: 528–534.

DE KROON, H., H. HUBER, J. F. STUEFER, AND J. M. VAN GROENENDAEL. 2005. A modular concept of phenotypic plasticity in plants. *New Phytologist* 166: 73–82.

DE KROON, H., E. J. W. VISSER, H. HUBER, L. MOMMER, AND M. J. HUTCHINGS. 2009. A modular concept of plant foraging behaviour: The interplay between local responses and systemic control. *Plant, Cell & Environment* 32: 704–712.

DREW, M. C., AND L. R. SAKER. 1975. Nutrient supply and the growth of the seminal root system in barley. II. Localized, compensatory increase in lateral root growth and rates of nitrate uptake when nitrate supply is restricted to only part of the root system. *Journal of Experimental Botany* 26: 79–90.

EINSMANN, J. C., R. H. JONES, P. MOU, AND R. J. MITCHELL. 1999. Nutrient foraging traits in 10 co-occurring plant species of contrasting life forms. *Journal of Ecology* 87: 609–619.

EISSENSTAT, D. M., AND R. D. YANAI. 1997. The ecology of root lifespan. *Advances in Ecological Research* 27: 1–60.

FRANSEN, B., H. DE KROON, C. G. F. DE KOVEL, AND F. VAN DER BOSCH. 1999. Disentangling the effects of root foraging and inherent growth rate on plant biomass accumulation in heterogeneous environment: A modeling study. *Annals of Botany* 84: 305–311.

FRIEND, A. L., M. R. EIDE, AND T. M. HINCKLEY. 1990. Nitrogen stress alters root proliferation in Douglas-fir seedlings. *Canadian Journal of Forest Research* 20: 1524–1529.

GERSANI, M., AND T. SACHS. 1992. Development correlations between roots in heterogeneous environments. *Plant, Cell & Environment* 15: 463–469.

GROSS, K. L., A. PETERS, AND K. S. PREGITZER. 1993. Fine root growth and demographic responses to nutrient patches in four old-field plant species. *Oecologia* 95: 61–64.

HARPER, J. L. 1977. Population biology of plants. Academic Press, San Diego, California, USA.

HODGE, A. 2004. The plastic plant: Root responses to heterogeneous supplies of nutrients. *New Phytologist* 162: 9–24.

HODGE, A. 2006. Plastic plants and patchy soils. *Journal of Experimental Botany* 57: 401–411.

HODGE, A. 2009. Root decisions. *Plant, Cell & Environment* 32: 628–640.

HU, F., AND P. MOU. 2013. Proliferation and growth of plant fine roots and the influences from nutrient variation: Implications from the split-root experiments of *Ailanthus altissima, Callistephus chinensis* and *Solidago canadensis. Chinese Journal of Plant Ecology* 37: 93–103 [in Chinese with English abstract].

JACKSON, R. B., AND M. M. CALDWELL. 1993. Geostatistical patterns of soil heterogeneity around individual perennial plants. *Journal of Ecology* 81: 683–692.

KING, J. S., T. J. ALBAUGH, H. L. ALLEN, M. BUFORD, B. R. STRAIN, AND P. DOUGHERTY. 2002. Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. *New Phytologist* 154: 389–398.

KOTLIAR, N. B., AND J. A. WIENS. 1990. Multiple scales of patchiness and patch structure: A hierarchical framework for the study of heterogeneity. *Oikos* 59: 253–260.

LAMB, E. G., J. J. HAAG, AND J. R. CAHILL. 2004. Patch–background contrast and patch density have limited effects on root proliferation and plant performance in *Abutilon theophrasti. Functional Ecology* 18: 836–843.

MAJDI, H. 2001. Changes in fine root production and longevity in relation to water and nutrient availability in a Norway spruce stand in northern Sweden. *Tree Physiology* 21: 1057–1061.

MALAMY, J. E. 2005. Intrinsic and environmental response pathways that regulate root system architecture. *Plant, Cell & Environment* 28: 67–77.

MCNICKLE, G. G., C. C. ST. CLAIR, AND J. F. CAHILL. 2009. Focusing the metaphor: Plant root foraging behaviour. *Trends in Ecology & Evolution* 24: 419–426.

MOU, P., R. J. MITCHELL, AND R. H. JONES. 1997. Root distribution of two tree species under a heterogeneous nutrient environment. *Journal of Applied Ecology* 34: 645–656.

MÜLLER, I., B. SCHMID, AND J. WEINER. 2000. The effect of nutrient availability on biomass allocation patterns in 27 species of herbaceous plants. *Perspectives in Plant Ecology, Evolution and Systematics* 3: 115–127.

NOVOPLANSKY, A., D. COHEN, AND T. SACHS. 1989. Ecological implications of correlative inhibition between plant shoots. *Physiologia Plantarum* 77: 136–140.

PREGITZER, K. S. 2002. Fine roots of trees—A new perspective. *New Phytologist* 154: 267–273.

PREGITZER, K. S., R. L. HENDRICK, AND R. FOGEL. 1993. The demography of fine roots in response to patches of water and nitrogen. *New Phytologist* 125: 575–580.

PRESTON, K. A., AND D. D. ACKERLY. 2004. The evolution of allometry in modular organisms. *In* M. Pigliucci and K. A. Preston [eds.], Phenotypic integration—Studying the ecology and evolution of complex phenotypes, 80–106. Oxford University Press, New York, New York, USA.

ROBERT, H. S., AND J. FRIML. 2009. Auxin and other signals on the move in plants. *Nature Chemical Biology* 5: 325–332.

RUFFEL, S., G. KROUK, D. RISTOVA, D. SHASHA, K. D. BIRNBAUM, AND G. M. CORUZZI. 2011. Nitrogen economics of root foraging: Transitive closure of the nitrate-cytokinin relay and distinct systemic signaling for N supply vs. demand. *Proceedings of the National Academy of Sciences, USA* 108: 18524–18529.

SCHEINER, S. M. 2001. MANOVA: Multiple responses variables and multispecies interactions. *In* S. M. Scheiner and J. Gurevitch [eds.], Design and analysis of ecological experiments, 99–115. Oxford University Press, New York, New York, USA.

SCHOETTLE, A. W., AND T. J. FAHEY. 1994. Foliage and fine root longevity of pines. *Ecological Bulletins* 43: 136–153.

SNOW, R. 1931. Experiments on growth and inhibition. Part II. New phenomena of inhibition. *Proceedings of the Royal Society of London, B, Containing Papers of a Biological Character* 108: 305–316.

STEWART, A. J. A., E. A. JOHN, AND M. J. HUTCHINGS. 2000. The world is heterogeneous: Ecological consequences of living in a patchy environment. *In* M. J. Hutchings, E. A. John, and A. J. A. Stewart [eds.], The ecological consequences of environmental heterogeneity, 1–8. Blackwell Science, Oxford, UK.

VON ENDE, C. 2001. Repeated-measures analysis: Growth and other time-dependent measures. *In* S. M. Scheiner and J. Gurevitch [eds.], Design and analysis of ecological experiments, 134–157. Oxford University Press, New York, New York, USA.

WANG, L. X., P. P. MOU, AND R. H. JONES. 2006. Nutrient foraging via physiological and morphological plasticity in three plant species. *Canadian Journal of Forest Research* 36: 164–173.

WATSON, M. A. 1986. Integrated physiological units in plants. *Trends in Ecology & Evolution* 1: 119–123.

WIJESINGHE, D. K., AND M. J. HUTCHINGS. 1999. The effects of environmental heterogeneity on the performance of *Glechoma hederacea*: The interactions between patch contrast and patch scale. *Journal of Ecology* 87: 860–872.

ZHANG, H., AND B. FORDE. 1998. An *Arabidopsis* MADS box gene that controls nutrient-induced changes in root architecture. *Science* 279: 407–409.

ZHANG, H., H. RONG, AND D. PILBEAM. 2007. Signaling mechanisms underlying the morphological responses of the root system to nitrogen in *Arabidopsis thaliana. Journal of Experimental Botany* 58: 2329–2338.