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3 Nutrient uptake by intact and disturbed roots of loblolly pine seedlings

4  
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1 **Abstract**

2 Most measurements of nutrient uptake use either hydroponic systems or soil-grown  
3 roots that have been disturbed by excavation. The first objective of this study was to  
4 test how root excavation affects nitrate uptake. Rates of  $\text{NO}_3^-$  uptake by mycorrhizal  
5 loblolly pine (*Pinus taeda* L.) seedlings were measured in intact sand-filled columns,  
6 hydroponics, and disturbed sand-filled columns. Total nitrate uptake in intact sand-  
7 filled columns was higher than in disturbed columns, indicating that disturbance  
8 lowers uptake. Transferring plants from the sand-filled columns to hydroponics had  
9 little effect on  $\text{NO}_3^-$  uptake beyond delaying uptake for an hour. The second  
10 objective of this study was to determine whether  $\text{NH}_4^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^+$  uptake  
11 could be studied using sand-filled columns, since previous studies had tested this  
12 method only for nitrate uptake. Uptake rates of  $\text{NH}_4^+$  and  $\text{K}^+$  were positive, while  
13  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  uptake rates were negative in intact sand-filled columns, indicating  
14 that net efflux may occur even without physical disturbance to the root system. The  
15 sand-filled column approach has some limitations, but holds promise for conducting  
16 nutrient uptake studies with minimal disturbance to the root system.

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25 **Key words:** root disturbance; efflux; ion uptake; loblolly pine seedling

## 1 **Introduction**

2 Nutrient uptake from solution culture has been used extensively to quantify uptake in  
3 laboratory experiments (e.g. Epstein *et al.*, 1963; Claasen and Barber, 1974;  
4 Marschner, 2002). In solution-culture systems, plants are often non-mycorrhizal,  
5 since growing ectomycorrhizal plants in hydroponics is difficult (Colpaert *et al.*,  
6 1999). Most plants in the field, however, are associated with mycorrhizal fungi,  
7 which have a significant impact on the mineral nutrition of plants (Smith and Read,  
8 1997). More recently, the solution culture method has been adapted to measure  
9 nutrient uptake by mycorrhizal tree seedlings by growing the seedlings in soil to  
10 allow mycorrhizal development and then transferring them to hydroponic solution for  
11 uptake measurements (Rygiiewicz *et al.*, 1984; Bledsoe and Rygiiewicz, 1986;  
12 Cumming, 1996; Constable *et al.*, 2001). This method has also been used in the  
13 field where roots are excavated from soil but left attached to the tree. The roots are  
14 placed in a nutrient solution, from which nutrient depletion is measured over time  
15 (Rennenberg *et al.*, 1996; BassiriRad *et al.*, 1999; Lucash *et al.*, 2005).

16       The problem with this approach is that removing the roots from the  
17 surrounding soil for uptake measurements may damage the roots and thereby  
18 reduce ion uptake. Although no studies to date have tested how excavating roots  
19 and transferring them to hydroponics affects their uptake rates, several studies have  
20 addressed how disturbance affects uptake. For example, gently rubbing roots can  
21 decrease their ATP content (Gronewald and Hanson, 1982), lower phosphorus influx  
22 (Gronewald and Hanson, 1982) and increase calcium influx (Rincon and Hanson,  
23 1986). Mechanically striking roots without causing any visible damage can cause a  
24 short-term decline in net nitrate uptake and an increase in nitrate efflux (Aslam *et al.*,  
25 1996). Our previous attempts to measure uptake of recently excavated, intact roots

1 resulted in considerable net efflux of some nutrients (McFarlane and Yanai, 2006,  
2 Lucash et al., 2007).

3         Excavating seedlings from soil also severs the extramatrical hyphae of  
4 mycorrhizae. Disrupting the extramatrical hyphae of vesicular-arbuscular  
5 mycorrhizae reduced P uptake by maize (McGonigle and Miller, 1996); no studies  
6 have addressed how excavation affects uptake by other nutrients or species.

7         In this study, we made use of a technique in which uptake is measured by  
8 monitoring the concentrations of nutrients in solution in a sand-filled column  
9 containing plant roots (Scholberg et al., 2002). This technique makes it possible to  
10 test how root excavation affects  $\text{NO}_3^-$  uptake by using a sequence of treatments to  
11 compare uptake from sand-filled columns with uptake in solution culture. In the first  
12 treatment, we measured  $\text{NO}_3^-$  uptake by mycorrhizal loblolly pine (*Pinus taeda* L.)  
13 seedlings in intact sand-filled columns. The second treatment was designed to  
14 measure the effect on root uptake of excavating the roots and severing mycorrhizal  
15 hyphae. It involved removing the seedlings from the sand-filled columns, placing the  
16 roots in nutrient solution and measuring their uptake using the hydroponic method.  
17 The two methods were repeated, which allowed us to determine how disturbance  
18 affects uptake in sand and to control for change over time in plant response.

19         The sand-filled column technique has been used to measure  $\text{NO}_3^-$  uptake by  
20 citrus seedlings (Scholberg et al., 2002) but has not been tested with other species  
21 or nutrients. Therefore we wanted to determine whether ammonium, calcium,  
22 magnesium and potassium uptake could be studied using this method. We  
23 measured  $\text{NH}_4^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^+$  uptake by loblolly pine seedlings using sand-  
24 filled columns and compared our uptake rates with those reported from other  
25 studies.

## 1 **Materials and Methods**

### 2 *Greenhouse Cultivation*

3 Loblolly pine seedlings were grown in a plantation for 1.5 yrs (East Tennessee  
4 Nursery, Delano, TN) before they were excavated and planted in sand-filled PVC  
5 pipes (10 cm inner diameter, 15 cm tall) closed at the bottom with landscaping fabric.  
6 Sand-filled columns without plants served as controls. Columns were placed in the  
7 greenhouse in Syracuse, NY from Jan. to Jun. 2003. The seedlings were exposed  
8 to naturally occurring airborne and soilborne inoculum.

9       Plants were grown with supplemental lighting for approximately 12 h per day.  
10 During the uptake measurement period, light levels in the greenhouse were  $360 \pm 58$   
11 (SE)  $\mu\text{mol photons m}^{-2} \text{ sec}^{-1}$  and average temperature was  $25.5 \pm 0.3$  °C. The  
12 columns were given 150 ml of water (approx. field capacity) daily. At harvest, the  
13 average total fresh seedling weight was  $68 \pm 3\text{g}$ .

14       Mycorrhizal fungi found on the surface of the roots were identified by DNA  
15 sequencing (Applied Biosystems Automated 3730xl DNA Analyzer, Cornell  
16 University). The DNA sequences were matched to species using blast searching in  
17 GENBANK (<http://www.ncbi.nlm.nih.gov/BLAST/>).

18

### 19 *Overview of Sand-Filled Column Method*

20 Prior to the beginning of the uptake measurements, 200 ml of dilute (0.05X)  
21 Hoagland's nutrient solution (Hoagland and Arnon, 1950) was added to loblolly pine  
22 and control (sand only) columns for one week. The morning of the measurements,  
23 we placed PVC caps with valves on the base of the sand-filled columns and linked  
24 them via tubing to a valve manifold, vacuum pump and reservoir (Scholberg et al.,  
25 2001). After closing the valves at the base of each column, 300 ml of solution was

1 added to each column. After one hour, the solution was removed by opening the  
2 valve and vacuuming each column at  $-30$  kPa for two minutes.

3 After removal of the initial solution, a period of nutrient uptake measurement  
4 commenced. We added to each column 300 ml of nutrient solution, slightly (10-20  
5 ml) more than field capacity. After one hour, the solution was vacuumed at  $-30$ kPa  
6 into a reservoir and the leachate was weighed. A subsample (8 ml) was removed  
7 and frozen until analysis. The remaining solution was reapplied to the columns and  
8 nutrient uptake measured at 3, 5, 7 and 24 h by repeating the procedure described  
9 above. To minimize the formation of depletion zones and anaerobic conditions  
10 during the sampling intervals, the columns were drained every 30 min by gravity and  
11 the solution re-applied. In addition, the solution was vacuumed and re-applied on an  
12 hourly basis.

13

#### 14 *Overview of Hydroponic Method*

15 For the hydroponic treatment, the seedlings and sand were removed from the  
16 columns. The seedlings were rinsed with DI water to remove any adhering sand and  
17 placed in Erlenmeyer flasks with 300 ml of dilute (0.05X) Hoagland's solution. The  
18 solution was aerated by pumping ambient air through tubing to pipet tips inserted in  
19 the flasks. Rubber stoppers were placed inside each flask to reduce the volume of  
20 solution and thereby maximize the ratio of root surface area to solution volume. Six  
21 flasks with solution and stoppers served as controls. Samples (8 ml) were  
22 withdrawn and flasks weighed to determine solution volume at 1, 3, 5, 7 and 24 h  
23 intervals.

24

## 1 *Disturbance Treatments*

2 To examine how disturbance affects nutrient uptake, plants were successively  
3 exposed to four treatments: (a) intact sand-filled columns, (b) hydroponics 1, (c)  
4 disturbed sand-filled columns, and (d) hydroponics 2. On the first day, we measured  
5 uptake of six plants using the sand-filled column method described above. This  
6 method allowed measurements of nutrient uptake by intact roots including the intact  
7 extramatrical hyphae of their mycorrhizal fungi. On the second day, we excavated  
8 the plants from the columns, placed them in aerated nutrient solution and measured  
9 uptake using the hydroponic method described above. This treatment simulated the  
10 transplant shock that occurs when intact roots are excavated and placed in nutrient  
11 solution. On day 3, seedlings were removed from hydroponic solution and re-  
12 planted into the sand-filled columns to determine if uptake by disturbed roots differs  
13 between nutrient solution and sand culture. On day 4, we re-excavated the plants,  
14 placed them into nutrient solution (hydroponics 2) and compared uptake to the  
15 previous hydroponic trial. This treatment allowed us to test for the effect of time or  
16 repeated experimentation on uptake. As a second measure of the effect of time, we  
17 measured uptake by a separate set of undisturbed plants (n=6) for four days.

18

## 19 *Uptake of $NH_4^+$ , $Ca^{2+}$ , $Mg^{2+}$ and $K^+$*

20 During the first disturbance experiment we measured uptake in intact sand-filled  
21 columns using dilute (0.05X) Hoagland's nutrient solution ( $225 \mu\text{mol L}^{-1} Ca^{2+}$ ,  $60$   
22  $\mu\text{mol L}^{-1} Mg^{2+}$ ,  $450 \mu\text{mol L}^{-1} K^+$  and  $100 \mu\text{mol L}^{-1} NH_4^+$ ). Sampling at 3, 5, 7, and 24  
23 hours was satisfactory for  $Ca^{2+}$ ,  $Mg^{2+}$  and  $K^+$ , but  $NH_4^+$  was depleted more rapidly.  
24 Therefore we conducted a follow-up experiment to measure  $NH_4^+$  uptake by a



1 separate set of seedlings (n=3) in sand-filled columns at higher concentrations  
 2 (0.14X Hoagland's, 950  $\mu\text{mol L}^{-1}$   $\text{NH}_4^+$ ) with sampling at 0.5, 1, 1.5, 2, 2.5, 3 and 4 h.

3

#### 4 *Laboratory Analyses and Uptake Calculations*

5 Nitrate and  $\text{NH}_4^+$  concentrations were determined by continuous flow analyzer  
 6 (Bran and Luebbe, AA3), and base cations ( $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$ , and  $\text{K}^+$ ) were analyzed using  
 7 ICP emission spectrometer (Spectro Analytical Instruments, FMA-03). Nutrient  
 8 uptake rates were calculated from changes over time in solution concentration (n=6  
 9 plants). We calculated uptake rates for each time interval by computing the change  
 10 in nutrient content of the solution (concentration times volume of leachate), taking  
 11 into account volume changes due to sample removal. To correct for other sources  
 12 and sinks of nutrients, the average change in nutrient content of controls at each  
 13 time interval was subtracted from the change in columns containing seedlings.  
 14 Recovery of nutrients in control columns was assessed by comparing nutrient  
 15 contents of original and leachate nutrient solutions.

16 At harvest, roots were severed from the shoots, cleaned and blotted dry.  
 17 Uptake rates were expressed on the basis of fresh root weight. Uptake kinetics of  
 18  $\text{NO}_3^-$  were estimated using a Michaelis-Menten model. The slope ( $I_n$ ) of the  
 19 depletion vs. time curve was calculated for each time period and then fit to

$$20 \quad I_n = \frac{I_{max}(C_0 - C_{min})}{K_m + (C_0 - C_{min})}$$

21 where  $I_{max}$  is the maximum ion influx,  $K_m$  is the solution concentration at  $\frac{1}{2} I_{max}$ ,  $C_0$  is  
 22 the ion concentration, and  $C_{min}$  is the ion concentration when  $I_n$  is zero.

23 To determine how the methods for measuring  $\text{NO}_3^-$  uptake (intact columns,  
 24 hydroponics 1, disturbed columns, hydroponics 2) affected uptake rates, we

1 analyzed the 7-hr cumulative uptake in a repeated measures ANOVA with time as  
2 the repeated measure (SAS Institute, 1985). Since the interaction of time and  
3 treatment was significant at  $\alpha = 0.05$ , we compared how the treatments varied with  
4 time using Student's multiple comparisons test. Within each treatment, we used  
5 linear regression to describe the relationship between uptake rate and nutrient  
6 concentration for  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$ , and  $\text{K}^+$ .

7

## 8 **Results**

### 9 *Identification of mycorrhizal fungi*

10 One simple, yellow morphotype with a thin mantle was found on all roots. DNA  
11 sequencing revealed that the fungus was *Wilcoxina*, which is known to establish  
12 mycorrhizal associations with loblolly pine in disturbed sites or in greenhouses.

13

### 14 *Evaluation of Sand-Filled Column Method for $\text{NO}_3^-$ Uptake*

15 We found that the  $\text{NO}_3^-$  concentration in the control columns was nearly constant  
16 over the 24-h period and consistently higher than the concentrations in the columns  
17 containing seedlings (Figure 1). Little of the applied  $\text{NO}_3^-$  remained in the column  
18 after vacuuming, as indicated by  $94\% \pm 0.7\%$  (SE) recovery of the applied  $\text{NO}_3^-$  in  
19 the control columns.

20

### 21 *Intact vs. Disturbed Columns*

22 To evaluate the effect of excavation on uptake rates in sand, we compared uptake in  
23 sand-filled columns measured on the first day with uptake by these same plants after  
24 they were excavated and repotted back into sand-filled columns on the third day.

25 We predicted that the physical disturbance associated with excavating the seedlings  
26 and severing their extramatrical hyphae would negatively affect  $\text{NO}_3^-$  uptake.

1 As expected, disturbance lowered  $\text{NO}_3^-$  uptake (Figure 2). At 7 hours,  
2 cumulative nitrate uptake was  $10.6 \mu\text{mol gfw}^{-1}$  in the intact sand-filled columns, while  
3 uptake was only  $2.8 \mu\text{mol gfw}^{-1}$  in the disturbed columns. By the end of the 24-h  
4 experiment, rates had slowed considerably in the intact columns, presumably  
5 because of the much lower concentrations attained ( $78 \pm 29 \mu\text{mol L}^{-1}$ , Figure 1).  
6 After being disturbed, plants depleted the solution to only  $312 \pm 74 \mu\text{mol L}^{-1}$  in the  
7 24-h period (Figure 1). These results indicate that disturbance lowers the ability of  
8 plants to take up  $\text{NO}_3^-$ .

9 Concentrations in solution changed over the course of these experiments,  
10 due to uptake (or efflux) by the plants. Using the observed concentrations, we can  
11 describe how uptake varied with concentration. On an individual plant basis, three of  
12 the six plants showed Michaelis-Menten saturation (data not shown). Figure 2  
13 shows uptake as a function of concentration, with the initial (and highest)  
14 concentration on the right, and the observations progressing over time to the left. In  
15 the intact columns, average nitrate uptake was positively related to concentration ( $p$   
16  $< 0.0001$ ). In the disturbed column treatment, plants had consistently low uptake  
17 rates, and thus showed little relationship of uptake to concentration ( $p = 0.8$ ).

18

### 19 *Intact Columns vs. Hydroponic 1*

20 Since seedlings are commonly excavated from soil and then transferred to  
21 hydroponics to measure uptake rates, we compared  $\text{NO}_3^-$  uptake between intact  
22 sand-filled columns and the Hydroponic 1 treatment, which we applied the following  
23 day.

24 The transfer of plants from sand culture to hydroponics initially caused a delay  
25 in  $\text{NO}_3^-$  uptake (Figure 2). Uptake was higher in the intact columns than hydroponics

1 at 1 h (1.0 vs. 0.2  $\mu\text{mol g fwt}^{-1} \text{ h}^{-1}$ ). After the first hour, uptake rates were similar  
2 between plants in undisturbed intact columns and plants in hydroponics.

3 Because uptake rates were initially low, uptake was not related to  
4 concentration in this treatment ( $p = 0.4$ , Figure 2). The highest rates of uptake were  
5 observed in the second and fourth sampling intervals, which resulted in an erratic  
6 pattern of uptake with concentration (Figure 2).

7

### 8 *Temporal Trends in Uptake*

9 Since our experiments took several days to conduct, we repeated the hydroponic  
10 treatment to test whether uptake of our plants was declining over the duration of the  
11 experiments, independent of the nature of the treatments. Hydroponics 2 resulted in  
12 lower uptake than hydroponics 1 (Figure 2). Reduced uptake could result from  
13 additional damage to the roots as they were transferred into and out of the disturbed  
14 column treatment, or uptake could be declining over the four days of the  
15 experiments, independent of our handling of them. To test whether  $\text{NO}_3^-$  uptake  
16 declined over time in undisturbed plants, we measured uptake by an additional set of  
17 six plants in undisturbed sand-filled columns for four days. Average uptake was  
18 similar across days, but it was higher than for plants in undisturbed columns in the  
19 disturbance experiment (0.9  $\mu\text{mol gfw}^{-1} \text{ h}^{-1}$  compared to 0.3  $\mu\text{mol gfw}^{-1} \text{ h}^{-1}$ ),  
20 probably because we used different plants. Variability in nitrate uptake was only  
21 0.12  $\mu\text{mol gfw}^{-1} \text{ h}^{-1}$  (SE) among plants across the four-day period. We conclude that  
22 the difference between hydroponics 1 and hydroponics 2 was due to the repeated  
23 disturbance to the roots rather than the duration of the experiment.

24

### 1 *Evaluation of Sand-Filled Column Method for NH<sub>4</sub><sup>+</sup> Uptake*

2 Analysis of NH<sub>4</sub><sup>+</sup> concentrations in the undisturbed columns revealed rapid declines  
3 in the controls (data not shown). As a result, we measured uptake of NH<sub>4</sub><sup>+</sup> at shorter  
4 time frames (0-1 h) than NO<sub>3</sub><sup>-</sup> (2 h) and at higher concentrations (950 μmol L<sup>-1</sup>) than  
5 earlier experiments (100 μmol L<sup>-1</sup>). Under these conditions, the sand-filled column  
6 method showed high recovery of NH<sub>4</sub><sup>+</sup>, with control recoveries consistently  
7 averaging 94%. Plants depleted NH<sub>4</sub><sup>+</sup> in the columns, compared to the controls  
8 (Figure 3). Plant uptake of NH<sub>4</sub><sup>+</sup> was not significantly related to concentration ( $p =$   
9 0.60, Figure 4), unlike uptake of NO<sub>3</sub><sup>-</sup>, which declined as concentrations declined ( $p$   
10  $< 0.0001$ , Figure 2). Ammonium uptake rates over the first 4 hours were  
11 approximately 1.4 times higher than NO<sub>3</sub><sup>-</sup> uptake rates on a molar basis at similar  
12 concentrations (Figures 2 and 3).

13

### 14 *Evaluation of Sand-Filled Column Method for Uptake of Base Cations*

15 The undisturbed sand-filled columns showed high recovery of base cations in the  
16 controls; average recovery was 94% for Ca<sup>2+</sup>, 93% for Mg<sup>2+</sup> and 93% for K<sup>+</sup>. The  
17 concentration of Ca<sup>2+</sup> and Mg<sup>2+</sup> was higher in the columns with plants than the  
18 controls (data not shown), due to high efflux rates by the plants during the first 3  
19 hours (Figure 5). Subsequently, uptake was positive and concentrations declined  
20 over time. The concentration of K<sup>+</sup> was also higher in columns with plants than the  
21 controls except in the last time interval, but this was due to water uptake by the  
22 plants (data not shown); K<sup>+</sup> uptake was consistently positive (Figure 5).

23

## 1 Discussion

2 Disturbing the soil-root system of loblolly pine seedlings reduced cumulative  $\text{NO}_3^-$   
3 uptake by 74%; plants had consistently lower rates in the disturbed than intact  
4 columns across the range of concentrations used (Figure 2). Other studies have  
5 shown that disturbance decreases  $\text{NO}_3^-$  uptake (Bloom and Sukrapanna, 1990) and  
6 increases  $\text{NO}_3^-$  efflux (Aslam et al., 1996). However, one study that used a  
7 disturbance regime similar to ours, whereby the researchers removed and  
8 homogenized the soil in the disturbed treatment, found that total N uptake of maize  
9 was higher in disturbed plants (McGonigle and Miller, 1996). Nitrogen mineralization  
10 rates may have increased in response to soil disturbance in their study, which would  
11 not be a problem in our experiment using sand.

12       Excavation of the root system may reduce uptake by physically damaging the  
13 roots or by disrupting uptake by mycorrhizal hyphae. In our study, as in others that  
14 excavated roots from soil and measured uptake (Rygiewicz et al., 1984; Gessler et  
15 al., 1998; BassiriRad et al., 1999), we could not distinguish the relative importance of  
16 root damage and mycorrhizal disruption in limiting uptake rates. By growing plants  
17 in nylon mesh cylinders that exclude roots but allow fungal hyphae to grow into the  
18 soil (Jasper et al., 1989), researchers have disrupted VAM hyphae without damaging  
19 the roots. This method has not yet been used in uptake experiments nor applied to  
20 ectomycorrhizal plants such as pines.

21       Since simply transferring roots between nutrient solutions can inhibit  $\text{NO}_3^-$   
22 uptake for 6 h (Bloom and Sukrapanna, 1990), we expected that transfer of roots  
23 from soil to hydroponics would significantly reduce  $\text{NO}_3^-$  uptake. Transferring roots  
24 from soil to hydroponics caused a delay in  $\text{NO}_3^-$  uptake (Figure 2), probably due to  
25 the disturbance associated with excavating the roots. After the first hour, uptake

1 rates of plants in hydroponics were similar to rates of roots with intact mycorrhizae in  
2 sand-filled columns (Figure 2). The absence of nutrient depletion zones in  
3 hydroponics may have compensated for the disruption of the uptake by extramatrical  
4 hyphae of mycorrhizae. These studies were conducted with loblolly pine in  
5 association with *Wilcoxina*, which is known to establish mycorrhizal associations with  
6 loblolly pine in disturbed sites or in greenhouses. The effects of disturbance on root  
7 uptake may differ with fungal species or strain.

8 Ammonium uptake rates measured using the sand-filled column method were  
9 similar to rates in most other studies. Ammonium uptake in our study (0.5 to 2  $\mu\text{mol}$   
10  $\text{gfw}^{-1} \text{h}^{-1}$ ) was similar to uptake by Norway spruce seedlings in sand culture (0.3  
11  $\mu\text{mol gfw}^{-1} \text{h}^{-1}$ , Eltrop and Marschner, 1996) and roots of Norway spruce (0.5  $\mu\text{mol}$   
12  $\text{gdwt}^{-1} \text{h}^{-1}$ , and beech (0.6  $\mu\text{mol gdwt}^{-1} \text{h}^{-1}$ , Gessler et al., 1998) trees that were  
13 excavated and measured in nutrient solution. Assuming the ratio of fresh:dry weight  
14 of fine roots is 9 based on data from fine roots of loblolly pine in the field  
15 (unpublished data), our  $\text{NH}_4^+$  uptake rates (10  $\mu\text{mol gdwt}^{-1} \text{h}^{-1}$ ) were similar to uptake  
16 rates by loblolly pine seedlings (10  $\mu\text{mol gdwt}^{-1} \text{h}^{-1}$ , Constable et al., 2001) and  
17 eastern deciduous tree seedlings (12  $\mu\text{mol gdwt}^{-1} \text{h}^{-1}$ , Lajtha, 1994) in solution  
18 culture. Excised poplar roots also had uptake rates (13  $\mu\text{mol gdwt}^{-1} \text{h}^{-1}$ , Rothstein et  
19 al., 2000) that were comparable to our roots. In contrast, Scots pine seedlings (35  
20  $\mu\text{mol gdwt}^{-1} \text{h}^{-1}$ , Boxman and Roelofs, 1987) and taega seedlings (20  $\mu\text{mol gdwt}^{-1}$   
21  $\text{h}^{-1}$ , Chapin et al., 1986) in solution culture had higher uptake rates than our loblolly  
22 pine roots.

23 Of the base cations, we observed positive uptake rates for  $\text{K}^+$  but not  $\text{Ca}^{2+}$  or  
24  $\text{Mg}^{2+}$  (Figure 5). Net uptake of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  was negative in Scots pine (Boxman  
25 and Roelofs, 1987), Douglas-fir, Sitka spruce, and western hemlock (Rygielwicz et

1 al., 1984) seedlings, except at high pH (Rygiewicz et al., 1984) and low  $\text{NO}_3^-$   
2 concentrations (Boxman and Roelofs, 1987). In previous field experiments, we  
3 observed negative uptake of  $\text{Mg}^{2+}$  in hardwoods but found uptake was positive in  
4 conifers using roots of mature trees that were excavated and measured in nutrient  
5 solution (Lucash et al., 2007). Uptake of  $\text{Ca}^{2+}$  was negative in chestnut and white  
6 oak but not in the other species we studied. Although we observed positive uptake  
7 of  $\text{K}^+$  in this study, we observed negative uptake rates of  $\text{K}^+$  by all species in our  
8 previous field experiments with roots of mature trees (Lucash et al., 2005, Lucash et  
9 al., 2007). Net uptake of  $\text{K}^+$  was also negative in Douglas-fir (Rygiewicz and  
10 Bledsoe, 1986, Rygiewicz et al., 1984), Sitka spruce (Rygiewicz et al., 1984),  
11 western hemlock (Rygiewicz et al., 1984) and Scots pine seedlings (Boxman and  
12 Roelofs, 1987). Even though net uptake rates are clearly not negative over the  
13 lifetime of the plant, efflux rates can exceed influx under certain experimental  
14 conditions. The timing of sampling (Scheurwater et al. 2000), plant nutritional status  
15 (Elliott et al. 1984; Oscarson et al. 1987; Clark et al. 2000), pretreatment nutrient  
16 concentrations (Rygiewicz and Bledsoe 1986) and ion interactions (Dean-Drummond  
17 and Glass 1983; Rygiewicz and Bledsoe 1986) can all affect whether net efflux  
18 occurs. Although we know that transient fluxes may occur, more studies using  
19 methods that minimize disturbance to the root system are needed to understand the  
20 relative importance of efflux under field conditions (Lucash et al., 2007).

21         There are some additional drawbacks to the measurement of nutrient uptake  
22 using sand-filled columns. First, growing plants in sand rather than soil is clearly  
23 artificial, but adsorption of nutrients makes soil an intractable medium. In preliminary  
24 experiments, we found that  $\text{NO}_3^-$  recovery rates were only  $83 \pm 14\%$  in a mixture of  
25 sand and potting soil (Lucash, 2005). Even using sand-filled columns, sampling



1 intervals and solution concentrations have to be chosen with care, as illustrated by  
2 our experience with adsorption of  $\text{NH}_4^+$ . Second, it is not possible to determine the  
3 exact concentration of nutrients at the root surface in sand as in solution culture,  
4 since concentrations will vary through the matrix as uptake (or efflux) occurs. We  
5 homogenized the concentrations every  $\frac{1}{2}$  to 1 h by recirculating the nutrient solution,  
6 but this mixing and vacuuming may disturb roots, mycorrhizas and microbes. Third,  
7 if nitrification rates differ between the plant and the control columns, estimates of  
8  $\text{NH}_4^+$  and  $\text{NO}_3^-$  uptake would be inaccurate. If these limitations can be overcome,  
9 the sand-filled column method may permit more accurate measurement of root  
10 uptake under field conditions than the hydroponic approaches.

11

## 12 **Conclusion**

13       The results of this study demonstrate that root excavation reduces  $\text{NO}_3^-$   
14 uptake measured in sand-filled columns. Transferring plants from sand-filled  
15 columns to hydroponics has little effect on  $\text{NO}_3^-$  uptake, suggesting that rates in  
16 hydroponics may be representative of rates observed in a soil matrix. Net uptake  
17 rates of Ca and Mg were negative in intact sand-filled columns, indicating that efflux  
18 rates may not be solely due to physical disturbance. Future studies should quantify  
19 efflux rates to more accurately estimate net uptake at the root scale. Unlike  
20 hydroponic studies which use excavated roots, the sand-filled column technique  
21 allows researchers to measure nutrient uptake with only minor disturbance to the  
22 root system.

23

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9

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- 6

## 1 **Figure Legends**

2

3 Figure 1. Nitrate depletion curves of controls ( $n = 6$ ) and loblolly pine seedlings ( $n =$   
4 6) exposed to four disturbance treatments. Vertical bars indicate standard errors.

5

6 Figure 2. Average net uptake of  $\text{NO}_3^-$  as a function of average concentration for  
7 loblolly seedlings exposed to four disturbance treatments ( $n = 6$ ). Uptake rates were  
8 determined from changes over time in solution concentration and volume. Vertical  
9 bars indicate the standard error of uptake; horizontal bars show the standard error of  
10 solution concentration.

11

12 Figure 3. Ammonium depletion curves of controls ( $n = 3$ ) and in intact sand-filled  
13 columns containing loblolly pine seedlings ( $n = 3$ ). Vertical bars indicate standard  
14 errors.

15

16 Figure 4. Average net uptake of  $\text{NH}_4^+$  as a function of average concentration for  
17 loblolly seedlings grown in intact sand-filled columns. Uptake rates were determined  
18 from changes over time in solution concentration and volume, measured using intact  
19 roots ( $n = 3$ ). Vertical bars indicate the standard error of uptake; horizontal bars  
20 show the standard error of solution concentration.

21

22 Figure 5. Average net uptake of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^+$  as a function of average  
23 concentration for loblolly seedlings grown in intact sand-filled columns. Negative  
24 numbers indicate net efflux. Uptake rates were determined from changes over time  
25 in solution concentration, measured using intact roots ( $n = 6$ ). Vertical bars indicate  
26 the standard error of uptake; horizontal bars show the standard error of solution  
27 concentration.