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Lucash, Melissa S.; Yanai, Ruth D.; and Joslin, J. Devereux, "Nutrient Uptake by Intact and Disturbed Roots of Loblolly Pine Seedlings" (2008). *Environmental Science and Management Faculty Publications and Presentations*. 109.

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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 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 NO_3^- uptake beyond delaying uptake for an hour. The second
10 objective of this study was to determine whether NH_4^+ , Ca^{2+} , Mg^{2+} and 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 NH_4^+ and K^+ were positive, while
13 Ca^{2+} and 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 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 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 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 NH_4^+ , Ca^{2+} , Mg^{2+} and 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 ± 58
11 (SE) $\mu\text{mol photons m}^{-2} \text{ sec}^{-1}$ and average temperature was 25.5 ± 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 $\mu\text{mol L}^{-1}$ 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 NH_4^+ concentrations were determined by continuous flow analyzer
 6 (Bran and Luebbe, AA3), and base cations (Ca^{+2} , Mg^{+2} , and 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 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 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 NO_3^- , NH_4^+ , Ca^{+2} , Mg^{+2} , and 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 NO_3^- Uptake*

15 We found that the 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 NO_3^- remained in the column
18 after vacuuming, as indicated by $94\% \pm 0.7\%$ (SE) recovery of the applied 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 NO_3^- uptake.

1 As expected, disturbance lowered 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 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 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 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 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 NH₄⁺ Uptake*

2 Analysis of NH₄⁺ concentrations in the undisturbed columns revealed rapid declines
3 in the controls (data not shown). As a result, we measured uptake of NH₄⁺ at shorter
4 time frames (0-1 h) than NO₃⁻ (2 h) and at higher concentrations (950 μmol L⁻¹) than
5 earlier experiments (100 μmol L⁻¹). Under these conditions, the sand-filled column
6 method showed high recovery of NH₄⁺, with control recoveries consistently
7 averaging 94%. Plants depleted NH₄⁺ in the columns, compared to the controls
8 (Figure 3). Plant uptake of NH₄⁺ was not significantly related to concentration ($p =$
9 0.60, Figure 4), unlike uptake of NO₃⁻, 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 NO₃⁻ 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 Ca²⁺, 93% for Mg²⁺ and 93% for K⁺. The
17 concentration of Ca²⁺ and Mg²⁺ 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⁺ 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⁺ uptake was consistently positive (Figure 5).

23

1 Discussion

2 Disturbing the soil-root system of loblolly pine seedlings reduced cumulative 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 NO_3^- uptake (Bloom and Sukrapanna, 1990) and
6 increases 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 (Rygielwicz 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 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 NO_3^- uptake. Transferring roots
24 from soil to hydroponics caused a delay in 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 μmol
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 μmol
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 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 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 K^+ but not Ca^{2+} or
24 Mg^{2+} (Figure 5). Net uptake of Ca^{2+} and 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 NO_3^-
2 concentrations (Boxman and Roelofs, 1987). In previous field experiments, we
3 observed negative uptake of 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 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 K^+ in this study, we observed negative uptake rates of 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 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 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 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 NH_4^+ and 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 NO_3^-
14 uptake measured in sand-filled columns. Transferring plants from sand-filled
15 columns to hydroponics has little effect on 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

1 **Acknowledgements**

2 We thank Johannes Scholberg for introducing us to the sand-filled column method
3 and helping troubleshoot our initial attempts to use it. Oscar Abelleira, Megan
4 Newhouse, Liz Schwartz, Don Bickelhaupt, Myriam Adam, and Adrienne Graham
5 provided technical assistance. We also thank Russ Briggs for use of his
6 autoanalyzer and Dave Eissenstat for his help in designing the disturbance
7 experiment. Financial support was provided by the National Science Foundation
8 through grants DEB-0087263 and 9211768.

9

1 References

- 2 Aslam, M., Travis, R.L., Rains, D.W. and Huffaker, R.C., 1996. Effect of root
3 perturbation and excision on nitrate influx and efflux in barley (*Hordeum*
4 *vulgare*) seedlings. *Physiol. Plant.* 97, 425-432.
- 5 BassiriRad, H.H., Prior, S.A., Norby, R.J. and Rogers, H.H., 1999. A field method of
6 determining NH_4^+ and NO_3^- uptake kinetics in intact roots: effects of CO_2
7 enrichment on trees and crop species. *Plant Soil* 217, 195-204.
- 8 Bledsoe, C.S. and Rygiewicz, P.T., 1986. Ectomycorrhizae affect ionic balance
9 during ammonium uptake by Douglas-fir roots. *New Phytol.* 102, 271-283.
- 10 Bloom, A.J. and Sukrapanna, S.S., 1990. Effects of exposure to ammonium and
11 transplant shock upon the induction of nitrate absorption. *Plant Physiol.* 94,
12 85-90.
- 13 Boxman, A.W. and Roelofs, J.G.M., 1987. Some effects of nitrate versus ammonium
14 nutrition on the nutrient fluxes in *Pinus sylvestris* seedlings. Effects of
15 mycorrhizal infection. *Can. J. Bot.* 66, 1091-1097.
- 16 Chapin, F.S.I., Van Cleve, K. and Tryon, P.R., 1986. Relationship of ion absorption
17 to growth rate in taiga trees. *Oecologia* 69, 238-242.
- 18 Claasen, N. and Barber, S.A., 1974. A method for characterizing the relation
19 between nutrient concentration and flux into roots of intact plants. *Plant*
20 *Physiol.* 54, 564-568.
- 21 Clark, G.T., Dunlop, J. and Phung, H.T., 2000. Phosphate absorption by *Arabidopsis*
22 *thaliana*: interactions between phosphorus status and inhibition by arsenate.
23 *Aust. J. Plant Phys.* 27, 959-965.

- 1 Colpaert, J.V., Van Tichelen, K.K., Van Assche, J.A. and Van Laere, A., 1999. Short-
2 term phosphorus uptake rates in mycorrhizal and non-mycorrhizal roots of
3 intact *Pinus sylvestris* seedlings. *New Phytol.* 143, 589-597.
- 4 Constable, J.V.H., BassiriRad, H., Lussenhop, J. and Zerihun, A., 2001. Influence of
5 elevated CO₂ and mycorrhizae on nitrogen acquisition: contrasting responses
6 in *Pinus taeda* and *Liquidambar styraciflua*. *Tree Physiol.* 21, 83-91.
- 7 Cumming, J.R., 1996. Phosphate-limitation physiology in ectomycorrhizal pitch pine
8 (*Pinus rigida*) seedlings. *Tree Physiol.* 16, 977-983.
- 9 Dean-Drummond, C.E. and Glass, A.D.M., 1983. Short term studies of nitrate uptake
10 into barley plants using ion-specific electrodes and ³⁶ClO₃⁻ II. Regulation of
11 NO₃⁻ efflux by NH₄⁺. *Plant Physiol.* 73, 105-110.
- 12 Elliott G.C., Lynch J. and Lauchli, A., 1984. Influx and efflux of P in roots of intact
13 maize plants. *Plant Physiol.* 76, 336-341.
- 14 Eltrop, L. and Marschner, H., 1996. Growth and mineral nutrition of non-mycorrhizal
15 and mycorrhizal Norway spruce (*Picea abies*) seedlings grown in semi-
16 hydroponic sand culture. I. Growth and mineral nutrient uptake in plants
17 supplied with different forms of nitrogen. *New Phytol.* 133, 469-478.
- 18 Epstein, E., Schmid, W.E. and Rains, D.W., 1963. Significance and technique of
19 short-term experiments on solute absorption by plant tissue. *Plant Cell*
20 *Physiol.* 4, 79-84.
- 21 Gessler, A., Schneider, S., Von Sengbusch, D., Weber, P., Hanemann, U., Huber,
22 C., Rothe, A., Kreutzer, K. and Rennenberg, H., 1998. Field and laboratory
23 experiments on net uptake of nitrate and ammonium by the roots of spruce
24 (*Picea abies*) and beech (*Fagus sylvatica*) trees. *New Phytol.* 138, 275-285.

- 1 Gronewald, J.W. and Hanson, J.B., 1982. Adenine nucleotide content of corn roots
2 as affected by injury and subsequent washing. *Plant Physiol.* 69, 1252-1256.
- 3 Hoagland, D.R. and Arnon, D.I., 1950. The water culture method of growing plants
4 without soil. California Agriculture Experiment Station.
- 5 Jasper, D.A., Abbott, L.K. and Robson, A.D., 1989. Soil disturbance reduces the
6 infectivity of external hyphae of vesicular-arbuscular mycorrhizal fungi. *New*
7 *Phytol.* 112, 93-99.
- 8 Lajtha, K., 1994. Nutrient uptake in eastern deciduous tree seedlings. *Plant Soil* 160,
9 193-199.
- 10 Lucash, M.S., Eissenstat, D.M., Yanai, R.D. and Joslin, J.D.. 2007. Estimating
11 nutrient uptake by mature tree roots under field conditions: challenges and
12 opportunities. *Trees* 21, 593-603.
- 13 Lucash, M.S., 2005. Methods for measuring nutrient uptake rates of intact roots of
14 seedlings and mature trees. PhD dissertation, Department of Forest and
15 Natural Resources Management. State University of New York College of
16 Environmental Science and Forestry, 114 p.
- 17 Lucash, M.S., Joslin, J.D. and Yanai, R.D., 2005. Temporal variation in nutrient
18 uptake capacity by intact roots of mature loblolly pine. *Plant Soil* 272, 253-
19 262.
- 20 Marschner, H., 2002. Mineral nutrition of higher plants. Academic Press, London.
- 21 McFarlane, K.J. and Yanai, R.D., 2006. Measuring nitrogen and phosphorus uptake
22 by intact roots of mature *Acer saccharum* Marsh., *Pinus resinosa* Ait., and
23 *Picea abies* (L.) Karst. *Plant Soil* 279, 163-172.

- 1 McGonigle, T.P. and Miller, M.H., 1996. Development of fungi below ground in
2 association with plants growing in disturbed and undisturbed soils. *Soil Biol.*
3 *Biochem.* 28, 263-269.
- 4 Oscarson, P., Ingemarsson, B., Ugglas, M. and Larsson, C.M., 1987. Short-term
5 studies of NO₃⁻ uptake in *Pisum* using ¹³NO₃⁻. *Planta* 170, 550-555.
- 6 Rennenberg, H., Schneider, S. and Weber, P., 1996. Analysis of uptake and
7 allocation of nitrogen and sulfur compounds by trees in the field. *J. Exp. Bot.*
8 47, 1491-1498.
- 9 Rincon, M. and Hanson, J.B., 1986. Controls on calcium ion fluxes in injured or
10 shocked corn root cells: importance of proton pumping and cell membrane
11 potential. *Physiol. Plant.* 67, 576-583.
- 12 Rothstein, D.E., Zak, D.R., Pregitzer, K.S. and Curtis, P.S., 2000. Kinetics of
13 nitrogen uptake by *Populus tremuloides* in relation to atmospheric CO₂ and
14 soil nitrogen availability. *Tree Physiol.* 20, 265-270.
- 15 Rygiewicz, P.T. and Bledsoe, C.S., 1986. Effects of pretreatment conditions on
16 ammonium and nitrate uptake by Douglas-fir seedlings. *Tree Physiol.* 1, 145-
17 150.
- 18 Rygiewicz, P.T., Bledsoe, C.S. and Zasoski, R.J., 1984. Effects of ectomycorrhizae
19 and solution pH on [¹⁵N] nitrate uptake by coniferous seedlings. *Can. J. For.*
20 *Res.* 14, 893-899.
- 21 Scheurwater, I., Dunnebacke, M., Eising, R. and Lambers, H., 2000. Respiratory
22 costs and rate of protein turnover in the roots of a fast-growing (*Dactylis*
23 *glomerata* L.) and a slow-growing (*Festuca ovina* L.) grass species. *J. Exp.*
24 *Bot.* 51, 1089-1097.
- 25

- 1 Scholberg, J.M.S., Parsons, L.R., Wheaton, T.A. McNeal, B.L. and Morgan, K.T.,
- 2 2002. Soil temperature, nitrogen concentration, and residence time affect
- 3 nitrogen uptake efficiency in citrus. *J. Environ. Qual.* 31, 759-768.
- 4 Smith, S.E. and Read, D.J., 1997. *Mycorrhizal symbiosis*. Academic Press, San
- 5 Diego. 605 p.
- 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 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 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 Ca^{2+} , Mg^{2+} and 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.