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Abstract

Most measurements of nutrient uptake use either hydroponic systems or soil-grown roots that have been disturbed by excavation. The first objective of this study was to test how root excavation affects nitrate uptake. Rates of NO_3^- uptake by mycorrhizal loblolly pine (*Pinus taeda* L.) seedlings were measured in intact sand-filled columns, hydroponics, and disturbed sand-filled columns. Total nitrate uptake in intact sand-filled columns was higher than in disturbed columns, indicating that disturbance lowers uptake. Transferring plants from the sand-filled columns to hydroponics had little effect on NO_3^- uptake beyond delaying uptake for an hour. The second objective of this study was to determine whether NH_4^+, Ca^{2+}, Mg^{2+} and K^+ uptake could be studied using sand-filled columns, since previous studies had tested this method only for nitrate uptake. Uptake rates of NH_4^+ and K^+ were positive, while Ca^{2+} and Mg^{2+} uptake rates were negative in intact sand-filled columns, indicating that net efflux may occur even without physical disturbance to the root system. The sand-filled column approach has some limitations, but holds promise for conducting nutrient uptake studies with minimal disturbance to the root system.

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

Introduction

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

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

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

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

In this study, we made use of a technique in which uptake is measured by monitoring the concentrations of nutrients in solution in a sand-filled column containing plant roots (Scholberg et al., 2002). This technique makes it possible to test how root excavation affects NO_3^- uptake by using a sequence of treatments to compare uptake from sand-filled columns with uptake in solution culture. In the first treatment, we measured NO_3^- uptake by mycorrhizal loblolly pine (*Pinus taeda* L.) seedlings in intact sand-filled columns. The second treatment was designed to measure the effect on root uptake of excavating the roots and severing mycorrhizal hyphae. It involved removing the seedlings from the sand-filled columns, placing the roots in nutrient solution and measuring their uptake using the hydroponic method. The two methods were repeated, which allowed us to determine how disturbance affects uptake in sand and to control for change over time in plant response.

The sand-filled column technique has been used to measure NO_3^- uptake by citrus seedlings (Scholberg et al., 2002) but has not been tested with other species or nutrients. Therefore we wanted to determine whether ammonium, calcium, magnesium and potassium uptake could be studied using this method. We measured NH_4^+, Ca^{2+}, Mg^{2+} and K^+ uptake by loblolly pine seedlings using sand-filled columns and compared our uptake rates with those reported from other studies.

Materials and Methods

Greenhouse Cultivation

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

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

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

Overview of Sand-Filled Column Method

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

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

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

Overview of *Hydroponic Method*

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

Disturbance Treatments

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

Uptake of NH_4^+, Ca^{2+}, Mg^{2+} and K^+

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

separate set of seedlings (n=3) in sand-filled columns at higher concentrations (0.14X Hoagland's, 950 μmol L^{-1} NH_4^+) with sampling at 0.5, 1, 1.5, 2, 2.5, 3 and 4 h.

Laboratory Analyses and Uptake Calculations

Nitrate and NH_4^+ concentrations were determined by continuous flow analyzer (Bran and Luebbe, AA3), and base cations (Ca^{+2}, Mg^{+2}, and K^+) were analyzed using ICP emission spectrometer (Spectro Analytical Instruments, FMA-03). Nutrient uptake rates were calculated from changes over time in solution concentration (n=6 plants). We calculated uptake rates for each time interval by computing the change in nutrient content of the solution (concentration times volume of leachate), taking into account volume changes due to sample removal. To correct for other sources and sinks of nutrients, the average change in nutrient content of controls at each time interval was subtracted from the change in columns containing seedlings. Recovery of nutrients in control columns was assessed by comparing nutrient contents of original and leachate nutrient solutions.

At harvest, roots were severed from the shoots, cleaned and blotted dry. Uptake rates were expressed on the basis of fresh root weight. Uptake kinetics of NO_3^- were estimated using a Michaelis-Menten model. The slope (I_n) of the depletion vs. time curve was calculated for each time period and then fit to

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

where I_{max} is the maximum ion influx, K_m is the solution concentration at ½ I_{max}, C_0 is the ion concentration, and C_{min} is the ion concentration when I_n is zero.

To determine how the methods for measuring NO_3^- uptake (intact columns, hydroponics 1, disturbed columns, hydroponics 2) affected uptake rates, we

analyzed the 7-hr cumulative uptake in a repeated measures ANOVA with time as the repeated measure (SAS Institute, 1985). Since the interaction of time and treatment was significant at $\alpha = 0.05$, we compared how the treatments varied with time using Student's multiple comparisons test. Within each treatment, we used linear regression to describe the relationship between uptake rate and nutrient concentration for NO_3^-, NH_4^+, Ca^{+2}, Mg^{+2}, and K^+.

Results

Identification of mycorrhizal fungi

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

Evaluation of Sand-Filled Column Method for NO_3^- Uptake

We found that the NO_3^- concentration in the control columns was nearly constant over the 24-h period and consistently higher than the concentrations in the columns containing seedlings (Figure 1). Little of the applied NO_3^- remained in the column after vacuuming, as indicated by 94% $\pm$ 0.7% (SE) recovery of the applied NO_3^- in the control columns.

Intact vs. Disturbed Columns

To evaluate the effect of excavation on uptake rates in sand, we compared uptake in sand-filled columns measured on the first day with uptake by these same plants after they were excavated and repotted back into sand-filled columns on the third day. We predicted that the physical disturbance associated with excavating the seedlings and severing their extramatrical hyphae would negatively affect NO_3^- uptake.

As expected, disturbance lowered NO_3^- uptake (Figure 2). At 7 hours, cumulative nitrate uptake was 10.6 μmol $gfwt^{-1}$ in the intact sand-filled columns, while uptake was only 2.8 μmol $gfwt^{-1}$ in the disturbed columns. By the end of the 24-h experiment, rates had slowed considerably in the intact columns, presumably because of the much lower concentrations attained (78 ± 29 μmol L^{-1}, Figure 1). After being disturbed, plants depleted the solution to only 312 ± 74 μmol L^{-1} in the 24-h period (Figure 1). These results indicate that disturbance lowers the ability of plants to take up NO_3.

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

Intact Columns vs. Hydroponic 1

Since seedlings are commonly excavated from soil and then transferred to hydroponics to measure uptake rates, we compared NO_3^- uptake between intact sand-filled columns and the Hydroponic 1 treatment, which we applied the following day.

The transfer of plants from sand culture to hydroponics initially caused a delay in NO_3^- uptake (Figure 2). Uptake was higher in the intact columns than hydroponics

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

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

Temporal Trends in Uptake

Since our experiments took several days to conduct, we repeated the hydroponic treatment to test whether uptake of our plants was declining over the duration of the experiments, independent of the nature of the treatments. Hydroponics 2 resulted in lower uptake than hydroponics 1 (Figure 2). Reduced uptake could result from additional damage to the roots as they were transferred into and out of the disturbed column treatment, or uptake could be declining over the four days of the experiments, independent of our handling of them. To test whether NO_3^- uptake declined over time in undisturbed plants, we measured uptake by an additional set of six plants in undisturbed sand-filled columns for four days. Average uptake was similar across days, but it was higher than for plants in undisturbed columns in the disturbance experiment (0.9 µmol $gfwt^{-1}$ h^{-1} compared to 0.3 µmol $gfwt^{-1}$ h^{-1}), probably because we used different plants. Variability in nitrate uptake was only 0.12 µmol $gfwt^{-1}$ h^{-1} (SE) among plants across the four-day period. We conclude that the difference between hydroponics 1 and hydroponics 2 was due to the repeated disturbance to the roots rather than the duration of the experiment.

Evaluation of Sand-Filled Column Method for NH_4^+ Uptake

Analysis of NH_4^+ concentrations in the undisturbed columns revealed rapid declines in the controls (data not shown). As a result, we measured uptake of NH_4^+ at shorter time frames (0-1 h) than NO_3^- (2 h) and at higher concentrations (950 µmol L^{-1}) than earlier experiments (100 µmol L^{-1}). Under these conditions, the sand-filled column method showed high recovery of NH_4^+, with control recoveries consistently averaging 94%. Plants depleted NH_4^+ in the columns, compared to the controls (Figure 3). Plant uptake of NH_4^+ was not significantly related to concentration (p = 0.60, Figure 4), unlike uptake of NO_3^-, which declined as concentrations declined (p < 0.0001, Figure 2*).* Ammonium uptake rates over the first 4 hours were approximately 1.4 times higher than NO_3^- uptake rates on a molar basis at similar concentrations (Figures 2 and 3).

Evaluation of Sand-Filled Column Method for Uptake of Base Cations

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

Discussion

Disturbing the soil-root system of loblolly pine seedlings reduced cumulative NO_3^- uptake by 74%; plants had consistently lower rates in the disturbed than intact columns across the range of concentrations used (Figure 2). Other studies have shown that disturbance decreases NO_3^- uptake (Bloom and Sukrapanna, 1990) and increases NO_3^- efflux (Aslam et al., 1996). However, one study that used a disturbance regime similar to ours, whereby the researchers removed and homogenized the soil in the disturbed treatment, found that total N uptake of maize was higher in disturbed plants (McGonigle and Miller, 1996). Nitrogen mineralization rates may have increased in response to soil disturbance in their study, which would not be a problem in our experiment using sand.

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

Since simply transferring roots between nutrient solutions can inhibit NO_3^- uptake for 6 h (Bloom and Sukrapanna, 1990), we expected that transfer of roots from soil to hydroponics would significantly reduce NO_3^- uptake. Transferring roots from soil to hydroponics caused a delay in NO_3^- uptake (Figure 2), probably due to the disturbance associated with excavating the roots. After the first hour, uptake

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

Ammonium uptake rates measured using the sand-filled column method were similar to rates in most other studies. Ammonium uptake in our study (0.5 to 2 µmol $gfwt^{-1}$ h^{-1}) was similar to uptake by Norway spruce seedlings in sand culture (0.3 µmol $gfwt^{-1}$ h^{-1}, Eltrop and Marschner, 1996) and roots of Norway spruce (0.5 µmol $gdwt^{-1}$ h^{-1}, and beech (0.6 µmol $gdwt^{-1}$ h^{-1}, Gessler et al., 1998) trees that were excavated and measured in nutrient solution. Assuming the ratio of fresh:dry weight of fine roots is 9 based on data from fine roots of loblolly pine in the field (unpublished data), our NH_4^+ uptake rates (10 µmol $gdwt^{-1}$ h^{-1}) were similar to uptake rates by loblolly pine seedlings (10 µmol $gdwt^{-1}$ h^{-1}, Constable et al., 2001) and eastern deciduous tree seedlings (12 µmol $gdwt^{-1}$ h^{-1}, Lajtha, 1994) in solution culture. Excised poplar roots also had uptake rates (13 µmol $gdwt^{-1}$ h^{-1}, Rothstein et al., 2000) that were comparable to our roots. In contrast, Scots pine seedlings (35 µmol $gdwt^{-1}$ h^{-1}, Boxman and Roelofs, 1987) and taega seedlings (20 µmol $gdwt^{-1}$ h^{-1}, Chapin et al., 1986) in solution culture had higher uptake rates than our loblolly pine roots.

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

al., 1984) seedlings, except at high pH (Rygiewicz et al., 1984) and low NO_3^- concentrations (Boxman and Roelofs, 1987). In previous field experiments, we observed negative uptake of Mg^{2+} in hardwoods but found uptake was positive in conifers using roots of mature trees that were excavated and measured in nutrient solution (Lucash et al., 2007). Uptake of Ca^{2+} was negative in chestnut and white oak but not in the other species we studied. Although we observed positive uptake of K^+ in this study, we observed negative uptake rates of K^+ by all species in our previous field experiments with roots of mature trees (Lucash et al., 2005, Lucash et al., 2007). Net uptake of K^+ was also negative in Douglas-fir (Rygiewicz and Bledsoe, 1986, Rygiewicz et al., 1984), Sitka spruce (Rygiewicz et al., 1984), western hemlock (Rygiewicz et al., 1984) and Scots pine seedlings (Boxman and Roelofs, 1987). Even though net uptake rates are clearly not negative over the lifetime of the plant, efflux rates can exceed influx under certain experimental conditions. The timing of sampling (Scheurwater et al. 2000), plant nutritional status (Elliott et al. 1984; Oscarson et al. 1987; Clark et al. 2000), pretreatment nutrient concentrations (Rygiewicz and Bledsoe 1986) and ion interactions (Dean-Drummond and Glass 1983; Rygiewicz and Bledsoe 1986) can all affect whether net efflux occurs. Although we know that transient fluxes may occur, more studies using methods that minimize disturbance to the root system are needed to understand the relative importance of efflux under field conditions (Lucash et al., 2007).

There are some additional drawbacks to the measurement of nutrient uptake using sand-filled columns. First, growing plants in sand rather than soil is clearly artificial, but adsorption of nutrients makes soil an intractable medium. In preliminary experiments, we found that NO_3^- recovery rates were only 83 ± 14% in a mixture of sand and potting soil (Lucash, 2005). Even using sand-filled columns, sampling

intervals and solution concentrations have to be chosen with care, as illustrated by our experience with adsorption of NH_4^+. Second, it is not possible to determine the exact concentration of nutrients at the root surface in sand as in solution culture, since concentrations will vary through the matrix as uptake (or efflux) occurs. We homogenized the concentrations every ½ to 1 h by recirculating the nutrient solution, but this mixing and vacuuming may disturb roots, mycorrhizas and microbes. Third, if nitrification rates differ between the plant and the control columns, estimates of NH_4^+ and NO_3^- uptake would be inaccurate. If these limitations can be overcome, the sand-filled column method may permit more accurate measurement of root uptake under field conditions than the hydroponic approaches.

Conclusion

The results of this study demonstrate that root excavation reduces NO_3^- uptake measured in sand-filled columns. Transferring plants from sand-filled columns to hydroponics has little effect on NO_3^- uptake, suggesting that rates in hydroponics may be representative of rates observed in a soil matrix. Net uptake rates of Ca and Mg were negative in intact sand-filled columns, indicating that efflux rates may not be solely due to physical disturbance. Future studies should quantify efflux rates to more accurately estimate net uptake at the root scale. Unlike hydroponic studies which use excavated roots, the sand-filled column technique allows researchers to measure nutrient uptake with only minor disturbance to the root system.

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Figure Legends

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

Figure 2. Average net uptake of NO_3^- as a function of average concentration for loblolly seedlings exposed to four disturbance treatments (*n* = 6). Uptake rates were determined from changes over time in solution concentration and volume. Vertical bars indicate the standard error of uptake; horizontal bars show the standard error of solution concentration.

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

Figure 4. Average net uptake of NH_4^+ as a function of average concentration for loblolly seedlings grown in intact sand-filled columns. Uptake rates were determined from changes over time in solution concentration and volume, measured using intact roots (n = 3). Vertical bars indicate the standard error of uptake; horizontal bars show the standard error of solution concentration.

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