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Melissa S. Lucash
Portland State University, lucash@pdx.edu

Dave M. Eissenstat
Pennsylvania State University

J. Devereux Joslin
Belowground Forest Research

Karis J. McFarlane
Oregon State University

Ruth D. Yanai
SUNY College of Environmental Science and Forestry

Estimating nutrient uptake by tree roots under field conditions: challenges and opportunities

Melissa S. Lucash[1], Dave M. Eissenstat[2], J. Devereux Joslin[3], Karis J. McFarlane[4] and Ruth D. Yanai[1*]

[1]Department of Forest and Natural Resources, State University of New York College of Environmental Science and Forestry, Syracuse, NY 13210, USA; [2]Department of Horticulture, Pennsylvania State University, 218 Tyson Building, University Park, PA 16802, USA; [3] Belowground Forest Research, Apartado 104-5655, Santa Elena de Monteverde, Puntarenas, Costa Rica and [4]Department of Forest Engineering, 204 Peavy Hall, Oregon State University, Corvallis, OR 97331 USA

Author for correspondence:

*Ruth D. Yanai

[1]Department of Forest and Natural Resources Management

State University of New York College of Environmental Science and Forestry

Syracuse, NY 13210

Phone: 315-470-3955

FAX: 315-470-6954

Email: rdyanai@mailbox.syr.edu

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Summary

Nutrient uptake by tree roots is difficult to measure accurately under field conditions using existing methods. In this review, we discuss current techniques for measuring uptake at the root surface including excised root, depletion, lysimeter and isotopic tracer methods. Although many insights have been gained using these methods, each has drawbacks. Estimates of uptake are affected by the sampling scheme, experimental conditions, whether roots are excised or not, concentrations of ions, and the rate of efflux of ions. Two recently developed approaches, SUM column method and digital autoradiography, hold considerable promise for improving our estimates of uptake by tree roots. A greater focus on methods development is critical to more accurately measuring uptake under conditions representative of those in the field.

Key words: autoradiography, efflux, excision, ion uptake, nutrient concentration, sand culture

Introduction

Surprisingly little is known about rates of nutrient uptake by tree roots in the field, despite their importance for the growth and survival of trees. Root systems of trees are extensive, spatially variable, morphologically and physiologically heterogeneous, and often intertwined with those of other plants. These characteristics make it difficult to accurately measure uptake under conditions representative of those in the field. The most common approach to estimating nutrient uptake by trees has been to construct nutrient budgets; however, this technique does not provide any information on processes at the root scale. Measurements of specific root uptake or uptake capacity are required. In this study, we define specific root uptake as the rate of uptake of nutrients per unit root mass. We define uptake capacity as specific root uptake at non-limiting concentrations. Current techniques for measuring specific root uptake, such as excised roots, depletion, lysimeters and isotopic tracers, have provided valuable information, but still fall short of providing realistic and accurate estimates of rates of nutrient uptake by trees in the field.

In the past, specific root uptake has been measured primarily using the fine roots of agricultural crops or tree seedlings in solution culture. Results of these studies are difficult to extrapolate to the field because roots in solution differ in age, morphology and physiology from those grown in solid media or the field (Skene et al. 1998). Furthermore, the nature of the rhizosphere and the movement of nutrients to the root surface are different in soils than in solution

culture (Barber 1995). Finally, roots of seedlings in solution culture are seldom mycorrhizal (Van der Driessche 1971; Ingestad and Lund 1979; Bledsoe and Rains 1981), whereas many tree roots depend on mycorrhizas to supplement nutrient uptake (Smith and Read 1997).

Despite the drawbacks, scientists still commonly use the solution culture method because rates of uptake can be readily measured. In contrast, current techniques for use with mature trees, the focus of this review, are subject to numerous methodological problems. For example, roots are often excised or disturbed prior to measurements, which may artificially increase the loss of nutrients from roots and thereby reduce net uptake (Bloom and Caldwell 1988). In addition, estimates of uptake vary widely, depending on the timing of sampling and experimental conditions such as nutrient concentrations and experiment duration. Continued development of methods is critical to a better understanding of nutrient uptake by mature trees, and how it varies among species, through the growing season, at different stages of plant development, and under different soil conditions.

More realistic estimates of uptake would also be useful in the development of models of nutrient uptake by mature tree roots. Current models are often unable to even accurately predict uptake by seedlings (Kelly et al. 1992; Kelly et al. 1994; Van Rees et al. 1999; Kelly et al. 2001); yet the parameters used in these models, based upon data from experiments on seedlings, are used to simulate uptake by mature trees. There is little reason to expect that parameters measured

on seedlings will accurately reflect uptake by mature trees, especially since trees change physiologically as they age (Espeleta and Eissenstat 1998; Law et al. 2001). Measuring uptake using roots of mature trees under conditions representative of the field would be advantageous to modelers seeking to understand plant-soil interactions.

In this review, we will first describe methods currently used to measure specific uptake rates of roots of mature trees. We classify these methods into two categories: (1) methods in which roots are excavated before measurement and (2) methods in which undisturbed roots are measured in soil. In the second section, we focus on the methodological challenges to obtaining more realistic estimates of uptake. In the third section, we describe recent technological advances that hold promise for the future. Finally, we describe opportunities for future research, which ultimately may lead to technological improvements and a better understanding of uptake by tree roots in the field.

Methods used to measure uptake by trees

Uptake by disturbed roots

Excised root method

The excised root technique (Epstein et al. 1963a) is commonly used for measuring specific uptake by seedlings and trees in the laboratory and in the field. In this method, roots are excavated from the soil, excised, and sealed inside cheesecloth "teabags". The bags are placed in an aerated solution containing a radioactive or

stable tracer of the nutrient of interest. After periods ranging from 10 min to 2 h, the rate of uptake is determined by analyzing tracer accumulation in the root.

This method has been used extensively since the 1960's and has proven extremely valuable for characterizing the nutritional status of trees (Bowen 1970; Jones et al. 1994; Hogberg et al. 1998) and measuring ion uptake by roots (Epstein et al. 1963b; Huang et al. 1992). Studies with excised roots were considered superior to studies with intact roots, since excision eliminates interactions between the root and shoot, which could complicate interpretation of the results (Hoagland and Broyer 1936).

More recently, however, some scientists have expressed reservations about using excised roots, arguing that excavation and excision can alter root respiration and uptake (Saglio and Pradet 1980; Bloom and Caldwell 1988). Moreover, the excised root method estimates only gross influx, defined as the rate of entry of the ion into the root. To estimate net uptake, defined as the difference between influx and efflux, two tracers (e.g. ^{32}P and ^{33}P) must be used (Elliott et al. 1984), or the tracer method must be combined with the depletion method (Clark et al. 2000), which is described below. Therefore, rates obtained using excised roots may not provide realistic estimates of *in situ* uptake. Rather this method is most appropriate for use in comparative studies to determine which factors affect gross nutrient uptake (e.g. nutrient concentration, temperature, plant age), assuming excision does not have an interactive effect on the factor of interest. The use of

this method is also limited by the special handling and disposal procedures for radioactive tracers and the relatively high analytical costs of stable isotopes.

Depletion method

The depletion method offers a possible improvement over the excised root method in that isotopes are not required and the roots remain intact (Gessler et al. 1998; BassiriRad et al. 1999; Lucash et al. 2005). In this method, roots of mature trees (Gessler et al. 1998; Gessler et al. 2002; Lucash et al. 2005) or seedlings (Bhat 1982; Marschner et al. 1991; BassiriRad et al. 1997; Gessler et al. 1998; BassiriRad et al. 1999) are excavated without detaching them from the tree, and placed in aerated nutrient solutions. Alternatively, roots are pruned and allowed to re-grow for several months in plastic trays containing soil (Escamilla and Comerford 1998) or bags containing a sand-soil mixture (McFarlane and Yanai 2006) before they are placed in nutrient solution. In both techniques, the depletion of nutrients from solution is measured by periodically sampling the solution to compute the net uptake rate.

The most significant advantage of this technique is that the roots are still attached to the tree and can continue to transport carbon, water, and nutrients bi-directionally. Carbohydrate supply may be particularly important for ions such as nitrate and ammonium that require substantial energy for uptake (Bloom et al. 1989; Bloom et al. 1992). Another advantage of this technique is that both

analytical techniques and supplies can be inexpensive, and there are fewer handling restrictions than with techniques requiring radioisotopes.

There are several disadvantages to the depletion method. This method involves excavating roots, which can affect rates of uptake. For example, efflux is greater than influx in some field studies, although net uptake is clearly not negative over the lifetime of the plant. In the Methodological Challenges section, these problems are discussed in more detail.

Uptake by undisturbed roots in porous media

Both the excised root and the intact depletion methods characterize uptake by roots that have been removed from the soil. Excavation may damage the roots and sever the extramatrical hyphae of their fungal associates, causing a reduction in uptake. To eliminate the effects of disturbance on uptake, alternative approaches are needed.

Lysimeter method

In the lysimeter method, seedlings or small trees are grown in porous media in containers ranging in size from small pots (Colpaert et al. 1999) to large tanks (Weinbaum et al. 1994; Syvertsen and Smith 1996). The root systems may be innoculated with mycorrhizal fungi (Colpaert et al. 1999). Nutrient solution is added to the medium and removed for sampling using a peristaltic, vacuum or sump pump. The leachate is weighed and analyzed for nutrient concentration; the

differences in nutrient content between the initial solution and the leachate are used to compute uptake.

One of the main advantages of this technique is that it can be used to assess the relative contribution of mycorrhizas to nutrient uptake without disturbing the root system (Colpaert et al. 1999). This technique has been used to quantify nitrogen uptake of small citrus (Syvertsen and Smith 1996) and plum trees (Weinbaum et al. 1994) in large lysimeters in the field but uptake was expressed at the tree level (kg N/ tree). One of the main drawbacks to this method is that it cannot be used for large trees, since the entire root system is limited by the size of the container.

Isotopic tracer method

To measure uptake by intact roots using isotopic tracers, nutrient solution containing tracers is applied to pots containing sand (Cui and Caldwell 1997; Proe et al. 2000; Yoder and Caldwell 2002) or peat (Ohlund and Nasholm 2004). In the field, isotopes can be applied with fertilizer (Weinbaum and Van Kessel 1998; Dinkelmeyer et al. 2003) or injected into the soil (Caldwell et al. 1985). In both techniques, uptake is calculated by analyzing the amount of tracer in the root and shoot tissue. In some studies, uptake has been calculated at the root scale (Cui and Caldwell 1997; Yoder and Caldwell 2002; Ohlund and Nasholm 2004), while in others uptake has been expressed at the whole-plant level (Proe et al. 2000; Dinkelmeyer et al. 2003).

The tracer method is useful for measuring gross uptake by undisturbed mycorrhizal roots (Ohlund and Nasholm 2004) and distinguishing between uptake and remobilization of nutrients, such as nitrogen (Weinbaum and Van Kessel 1998; Proe et al. 2000) and potassium (Proe et al. 2000). The relative competitiveness of different species (Caldwell et al. 1985; Yoder and Caldwell 2002) and seasonal trends in uptake (Nambiar and Bowen 1986) can also be quantified using this method. This technique is difficult to apply to large trees in the field (Dinkelmeyer et al. 2003; McKane et al. 2003) due to problems of isotope dilution, sampling large plants and determining the concentration at the root surface.

Methodological Challenges

In this section, we describe the problems with existing methods to draw attention to the limits of our current techniques. These challenges need to be overcome to obtain more realistic estimates of uptake by trees.

Bias introduced by root sampling approaches

Selecting which roots to sample, when to sample and how long to sample are all critical decisions in uptake studies. Since these factors affect measured uptake rates, the method of selecting roots and the timing of sampling should be considered before implementing a study.

Most researchers measure uptake by young, fine diameter roots because they are considered to be most active in nutrient uptake. Older, thicker roots, however, may also take up nutrients and are a significant proportion of root biomass in mature trees. Depletion of NH_4^+ and magnesium was observed in the rhizosphere of both young and old roots of Norway spruce, indicating that uptake occurred in both ages of root (Dieffenbach et al. 1997). In cherry roots, phosphorus uptake was not statistically different between young (white) and old (woody) roots (Atkinson and Wilson 1979).

Diurnal variation in uptake should be considered when measuring uptake rates. Many studies with agricultural crops report diurnal rhythms of uptake of NO_3^- (Hansen 1980; Pearson et al. 1981; Scaife and Schloemer 1994; Delhon et al. 1996) and NH_4^+ (Ourry et al. 1996; Macduff et al. 1997). Only one study to date has examined diurnal patterns of uptake by trees (Gessler et al. 2002). In that study, the diurnal patterns of NH_4^+ uptake by mature trees were species-specific; spruce exhibited little diurnal fluctuations in rates, while beech had higher NH_4^+ uptake during the day than the night.

Uptake also varies seasonally, but selecting the best time to conduct experiments is problematic because the timing of uptake differs among species. Ammonium uptake by mature loblolly pine roots was higher in April than July (Lucash et al. 2005). In another study in which the depletion method was used, uptake of NH_4^+ by mature subalpine spruce and beech was higher in summer than spring (Gessler et al. 1998).

Seasonal patterns in uptake also vary from year to year. In a two-year study, uptake of phosphate by excised roots of balsam fir in April was two times higher in the first than the second year (Langlois and Fortin 1984). In another study, uptake of NH_4^+ by intact roots of spruce was similar in the first and second years, but uptake by intact beech roots was significantly higher in July and Sept of the second year (Gessler et al. 1998).

The duration of the experiment can have dramatic effects on estimates of uptake. In experiments with crop plants in which concentrations were kept constant, uptake varied significantly over time. Nitrate uptake dropped by 50% after the first 14 h, and potassium uptake decreased by 27% after 36 h (Glass et al. 1987). Therefore, the length of the experiment can affect uptake rates, and rates measured over different durations may not be comparable. Sampling intervals that have been used in experiments with seedlings and mature trees have ranged from 10 min (Lajtha 1994) to 30 min (Bhat 1982) for P, 15 min (Rothstein et al. 2000) to 1 day (Eltrop and Marschner 1996) for NO_3^- and NH_4^+, and 2 h (Lucash et al. 2005) to 1-2 days for K^+, calcium and Mg^{2+} (Bledsoe and Rains 1981). In experiments with long durations, hypoxic conditions may develop if the roots are not adequately aerated and uptake rates may be suppressed (Escamilla and Comerford 1998)

Effects of excision and disturbance on uptake are variable

Although the effects of excision on uptake have never been studied in tree seedlings or mature trees, the effects of excision on uptake have been shown to be ion-specific in agricultural crops. Excision has been shown to decrease NO_3^- (Bloom and Caldwell 1988; Aslam et al. 1996) and NH_4^+ uptake (Bloom and Caldwell 1988), root respiration (Saglio and Pradet 1980; Bloom and Caldwell 1988; but see Lipp and Andersen 2003) and carbohydrate supply (Clarkson et al. 1974; Saglio and Pradet 1980). In contrast, excision had no effect on P uptake in two studies (Gronewald and Hanson 1980; Gronewald and Hanson 1982). The effects of excision on K^+ and Ca^{+2} uptake are not well established. Excision of barley roots significantly decreased K^+ uptake in two studies (Glass 1978; Bloom and Caldwell 1988), but had no effect on K^+ uptake in another (Huang et al. 1992). Excision reduced Ca influx in corn (Rincon and Hanson 1986) but had no effect on uptake by barley (Clarkson et al. 1974).

Treatment differences within studies can be misinterpreted if excision has an interactive effect on the treatment of interest. For example, excision had a greater effect on uptake by barley at low than at high temperatures (Clarkson et al. 1974); thus, the effects of temperature on uptake may be difficult to evaluate using this method. The effects of excision are species-specific (Huang et al. 1992), which complicates the task of comparing treatment differences among species. The effects of excision on uptake have never been studied in tree species, as far as we know, but we can expect them to be equally complex.

Comparing results across studies using the excised root method is difficult because there is no standard protocol. For example, root segment length varies, even though influx rates per unit mass increase with segment length (Gronewald and Hanson 1980; Huang et al. 1992). In addition, the length of time the excised roots are kept in solution (also known as the "aging" effect) affects uptake rates (Glass 1978; Huang et al. 1992). For example, K^+ influx rates of excised barley roots increased by 50% between 1 h and 2 h (Glass 1978). Establishing methodological guidelines for the excised root method would make it easier to compare results across studies.

In both the excised root and the depletion method, roots of seedlings and mature trees are excavated from the soil before uptake is measured. In one study, roots of four tree species were exposed to pretreatments to reduce damage to the roots or allow roots to recover from the disturbance associated with excavating intact roots (McFarlane and Yanai 2006). Unexpectedly, roots given pretreatments did not have consistently higher uptake rates than roots recently disturbed, indicating that pretreatments designed to minimize disturbance may not be effective.

In another study, the effects of disturbance on uptake were studied by examining how uptake was affected by the transfer of seedlings to solution culture. In that study, NO_3^- uptake was similar between undisturbed mycorrhizal loblolly pine seedlings grown in sand-filled lysimeters and seedlings transferred to solution culture (Lucash et al. in review). This result may indicate that mycorrhizal hyphae

are not important for uptake of mobile nutrients such as NO_3^- (Eltrop and Marschner 1996). Alternatively, the negative effect on uptake of severing the extramatrical hyphae of mycorrhizas may have been masked by the positive effect of eliminating nutrient depletion zones in solution culture. More studies are necessary to distinguish the effects of root excavation from hyphal excision on nutrient uptake rates.

Underestimation of the importance of nutrient efflux

As described above, the use of tracers to measure uptake detects nutrient influx but not efflux. In some situations, the rate of efflux can be a significant component of net uptake, sometimes equaling or exceeding the rate of influx. In a study of *Arabidopsis* where both influx (using the ^{32}P method) and net uptake (using the depletion method) were quantified, influx of ^{32}P occurred almost immediately (Clark et al. 2000). Net uptake, however, was negative for 1-3 hours, indicating that efflux exceeded influx during that period. In a set of studies using the depletion method, we found that net uptake of NH_4^+, $Mg^{2+}$and Ca^{+2} over a 2 h period was positive for some tree species but not others; uptake of K^+ was consistently negative (Figure 1).

In the excised root and depletion methods, roots are severed or excavated just prior to measurements, which may cause high rates of efflux. McFarlane and Yanai (2006) tested whether various pretreatments would reduce damage to the roots or allow roots to recover from the disturbance associated with excavating

intact roots. Consistent net efflux of Ca^{+2}, Mg^{2+}, K^{+}, and Na^{+} was observed across treatment in four tree species simultaneously with positive net uptake of NH_4^{+}, NO_3^{-}, and PO_4^{3-} and Al^{3+} (Figure 2). Therefore the disturbance associated with excavation was not responsible for nutrient efflux.

The fact that net nutrient efflux occurs in roots that have been excavated suggests that either uptake rates or efflux rates are not realistic under these experimental conditions. The importance of measuring nutrient efflux has not been clearly recognized, perhaps because tracer methods estimate only gross influx. The need remains for methods that minimize disturbance and produce realistic estimates of efflux and net uptake.

Influences of nutrient concentration on estimates of uptake

A wide range of concentrations has been used to assess uptake rates of tree seedlings (Table 1), but the justification for the choice of concentration is seldom reported. In some studies, roots are exposed to solutions that simulate bulk soil solution concentrations (Rennenberg et al. 1996; Gessler et al. 1998), although concentrations at the root surface differ from bulk soil (Barber 1995). Concentrations at the root surface can be measured, but the methods used are often destructive (Gobran and Clegg 1996; Bakker et al. 1999) and produce concentrations inconsistent with nutrient uptake models (Yanai et al. 2003). New methods that use non-destructive *in situ* sampling to characterize concentrations at the root surface (Dieffenbach et al. 1997; Dieffenbach and Matzner 2000) hold

promise for obtaining realistic estimates of concentrations at the root surface. These techniques may be valuable for selecting concentrations to use in laboratory experiments or improving model estimates of uptake rates. Nutrient concentrations should be carefully selected to facilitate the comparisons of results across studies and to obtain estimates of uptake using concentrations that are representative of field conditions.

Nutrient uptake is often measured at a range of nutrient solution concentrations and then fitted to the following Michaelis-Menten equation:

$$U = \frac{V_{max} * (C_0 - C_{min})}{K_m + (C_0 - C_{min})}$$

where U is the uptake rate (amount per unit time per unit root), V_{max} is the maximum uptake rate (or uptake capacity) at high concentration (same units as U), C_0 is the concentration at the root surface, K_m is the concentration at which uptake is ½ V_{max} and C_{min} is the concentration below which uptake ceases (Claasen and Barber 1974). In most studies with trees (Eltrop and Marschner 1996; Rothstein et al. 1996; BassiriRad et al. 1999; Rothstein et al. 2000; Hangs et al. 2003), however, the formula is applied to data without reporting model fit or addressing whether another model might better describe the data. Depending on the concentrations used in the study, uptake may be linearly related to concentration, as observed for P uptake in red maple (Kelly and Kelly 2001) and NO_3^- uptake in loblolly pine (Lucash et al. 2005).

In addition, internal nutrient concentrations can affect estimates of kinetic parameters. Plants have high uptake capacity following a period of nutrient deficiency (Lee and Rudge 1986; Siddiqi et al. 1989), and low uptake capacity after exposure to high concentrations, due to saturation of exchange sites at the root surface (Dean-Drummond 1982; Siddiqi et al. 1990). In one study, uptake rates of NH_4^+ by Douglas-fir were reduced by high pretreatment concentrations of K^+, and K^+ uptake rates were reduced by high pretreatment concentrations of NO_3^- (Rygiewicz and Bledsoe 1986).

In agricultural crops, uptake of NO_3^-, NH_4^+ and PO_4^{-3} has been observed to conform to the Michaelis-Menten model at low but not high concentrations (Siddiqi et al. 1990; Aslam et al. 1992; Wang et al. 1993; Barber 1995). This multiphasic pattern indicates that there are distinct uptake systems, which differ in their affinity for nutrients. The number of uptake systems not only varies with species (Nissen 1991) and variety (Woodend et al. 1986), but also with the pretreatment concentrations (Drew et al. 1984). Future studies should determine whether uptake is multiphasic in mature trees and seedlings at concentrations normally found in the field.

Studies measuring uptake are often conducted by studying the uptake of one ion at a time. In nature, as well as under experimental conditions, however, roots are exposed to multiple ions simultaneously, and the presence of one ion can affect uptake of another. For example, the presence of NH_4^+ inhibits NO_3^- uptake by Norway spruce (Marschner et al. 1991), K^+ inhibits NH_4^+ uptake by

spruce and barley but not by rice (Wang et al. 1996), and aluminum inhibits Ca^{+2}, NH_4^+, and K^+ uptake and enhances influx of NO_3^- and PO_4^{-3} by barley (Nichol et al. 1993). Some depletion studies have exposed roots to solutions containing multiple ions at concentrations that attempt to simulate soil solution concentrations (Rennenberg et al. 1996; Gessler et al. 1998; Lucash et al. 2005). If the rate of uptake differs among nutrients, however, the ratios of nutrients may change over the duration of the experiment, creating artificial conditions. Studies should expose roots to multiple ions at concentrations representative of field conditions to produce realistic estimates of uptake capacity.

Recent Methodological Advances

Current methods for measuring specific uptake by tree roots have many drawbacks, as outlined above. Two new approaches hold considerable promise for improving our estimates of uptake by tree roots.

Soil uptake monitoring (SUM) method

The soil uptake monitoring (SUM) method is a type of lysimeter method developed to measure NO_3^- uptake of seedlings of woody plants (Scholberg et al. 2001; Lucash 2005). In this method, seedlings are grown in sand-filled PVC columns in the laboratory or in the field. To measure uptake, the valves at the base of each column are closed and nutrient solution of known volume and concentration is added to the columns. To collect the solution, the valve is opened and a vacuum

is applied to drain the solution into the reservoirs. The solution can be sampled at this point (Scholberg et al. 2001) or it can be repeatedly reapplied to minimize the formation of depletion zones around the roots (Lucash et al. in review). At each sampling interval, the leachate is weighed and analyzed for nutrient concentration; the differences in nutrient content between the initial solution and the leachate is used to compute uptake.

The SUM column method has been used to quantify uptake of NO_3^- by citrus (Scholberg et al. 2001) and uptake of NO_3^- and NH_4^+ by loblolly pine (Lucash et al. in review). This method was also used to measure diurnal trends in NO_3^- and NH_4^+ uptake by white pine seedlings; variation between days was greater than diurnal variation (Figure 3).

To our knowledge, this is the first technique that allows specific uptake rates to be estimated in soil without excavating the roots. A key advantage over the solution culture method is that mycorrhizas and microbes are better able to establish in sand than solution. Most tree roots are mycorrhizal in the field (Smith and Read 1997) and the presence of mycorrhizas affects the uptake kinetics of trees (Eltrop and Marschner 1996; Constable et al. 2001).

Although it has been used only with seedlings to date, the SUM column method could be applied to roots of mature trees. At two field sites in Syracuse, NY, we excavated small soil pits and installed capped SUM columns vertically in the soil. We attached tubing to the cap and made sure the tubing extended slightly above the soil surface. We trained root branches of eastern hemlock and

sugar maple to grow into the side of the column through a small hole. Unfortunately fresh root weights in the columns averaged less than 1 g after 4 months, and we were unable to detect differences in uptake between controls and plants. More studies are necessary to test methods to promote root growth in the SUM columns. The columns may need to be watered or fertilized to promote root growth, since the surrounding soil is likely to be more nutrient-rich than the medium in the columns.

Although the SUM column method holds promise for obtaining more realistic estimates of uptake by seedlings and mature trees, there are several drawbacks to this technique. First, as with other lysimeter methods, plants must be grown in sand rather than native soil to minimize adsorption of nutrients on the soil surface. However, uptake in sand may not be representative of uptake in native soil. Second, it is more difficult to determine the concentration of nutrients at the root surface in sand than solution culture since nutrient solution can more easily be mixed and aerated when not in a soil matrix. We homogenized the solution concentrations every ½ to 1 h by recirculating the nutrient solution, but this mixing may disturb roots, mycorrhizas and microbes in the sand. Other lysimeter studies use small containers (25 cm-long glass vials) with small seedlings (dry weights < 4 g) to maximize the availability of nutrients at the root surface (Colpaert et al. 1999). Third, differences in nitrification rates in the plant and the control SUM columns would cause estimates of NH_4^+ and NO_3^- uptake to be inaccurate.

Spatial mapping methods

A promising technique for determining which roots are actively taking up nutrients is the digital autoradiographic technique (Rubio et al. 2004). In this technique, plants are grown in sand-filled pots in the laboratory and irrigated with nutrient solution. To measure uptake, root systems are excavated but left attached to the shoot and transferred into containers with ^{32}P-labelled solution. After uptake occurs, the roots are removed, excised and separated into different root classes. The root segments are scanned to measure surface area and length and placed on a phosphor screen that generates a graphical representation of the spatial distribution of ^{32}P. The rate of ^{32}P uptake is used to estimate specific uptake rates, as with other labeling methods.

This is the first technique to quantify how P uptake rates vary within a root system using intact plants. In the study, P uptake differed in basal, lateral basal, and lateral tap roots of bean plants (Rubio et al. 2004). Spatial variation in uptake along the root axis is important for understanding how plants regulate uptake and for improving uptake models, which currently do not address spatial heterogeneity in uptake within a root system (Smethurst and Comerford 1993). Like other methods that rely on uptake of tracers, this method can estimate only gross influx rates.

Conclusions

Measuring specific root uptake by intact roots of trees is critical to obtaining realistic rates of uptake and improving our understanding of belowground processes. The most widely used methods to measure uptake, however, rely on measurements of uptake by tree seedlings in solution culture. Methods that have measured uptake by intact roots have relied upon observations from roots that have been excised or excavated from the soil. Although these estimates of uptake may be useful for comparative purposes, more realistic estimates are necessary to describe root uptake by seedlings and mature trees in the field.

The task of measuring specific root uptake is limited by methodological problems which need to be addressed. Special consideration should be given to selecting the best time to sample and the concentrations to which the root will be exposed. Uptake measurements that exclude efflux should be interpreted with caution. In addition, the amount of disturbance should be carefully evaluated before selecting a method since many methods rely on observations from excised or excavated roots. Studies that compare estimates of uptake among methods are essential to determine how different experimental conditions affect estimates of uptake.

Recent developments using sand culture and digital autoradiography show promise, since they enable researchers to quantify uptake of intact root systems. These new methods create opportunities to more accurately measure spatial and diurnal variation in uptake rates of seedlings and mature trees in the field.

Although considerable progress has been made in the field of nutrient uptake by trees, many questions about how and when tree roots take up nutrients remain unanswered. How does uptake vary diurnally and seasonally for a given species or forest type? How much of that variation is controlled by nutrient availability as opposed to phenology of the tree? How much do mycorrhizas contribute to nutrient acquisition under field conditions for various tree species? Such questions not only are critical to our mechanistic understanding of nutrient acquisition, but also have practical implications for maximizing fertilizer use efficiency and forest production.

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1 Table 1. Ammonium concentrations used to quantify uptake capacity in nine

2 studies involving tree species, in order of increasing concentration.

Species	Conc. ($\mu mol\ L^{-1}$)	Technique	Author
Fagus sylvatica *Picea abies*	53-55	Depletion	Gessler et al. (1998)
Picea abies	0-150	Depletion	Marschner et al. (1991)
Acer rubrum *Acer saccharum*	0-200	Depletion	BassiriRad et al. (1999)
Pinus ponderosa *Pinus taeda*	0-500	Excised	BassiriRad et al. (1996)
Populus tremuloides	0-500	Excised	Rothstein et al. (2000)
Picea abies	800	Lysimeter	Eltrop and Marschner (1996)
Acer saccharum	0-1000	Excised	Rothstein et al. (1996)
Betula alleghaniensis *Carya ovata* *Fagus sylvatica* *Fraxinus americana* *Liriodendron tulipifera* *Prunus serotina* *Quercus phellos*	0-4000	Excised	Lajtha (1994)

3

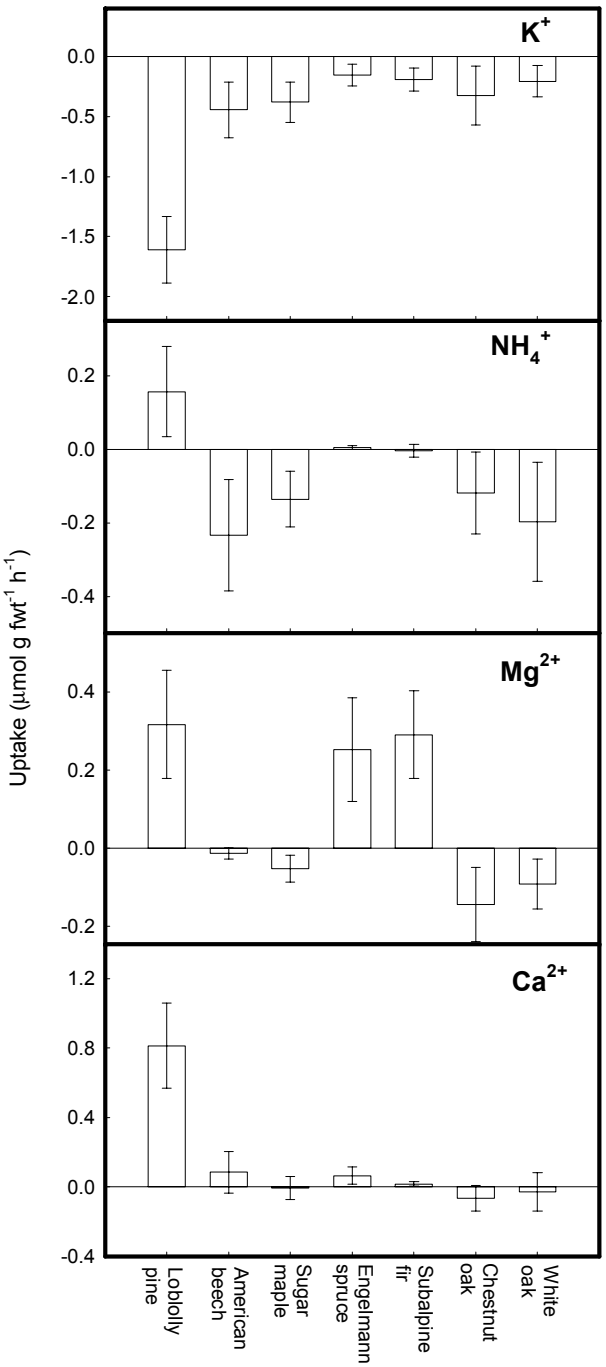
4

Figure Legends

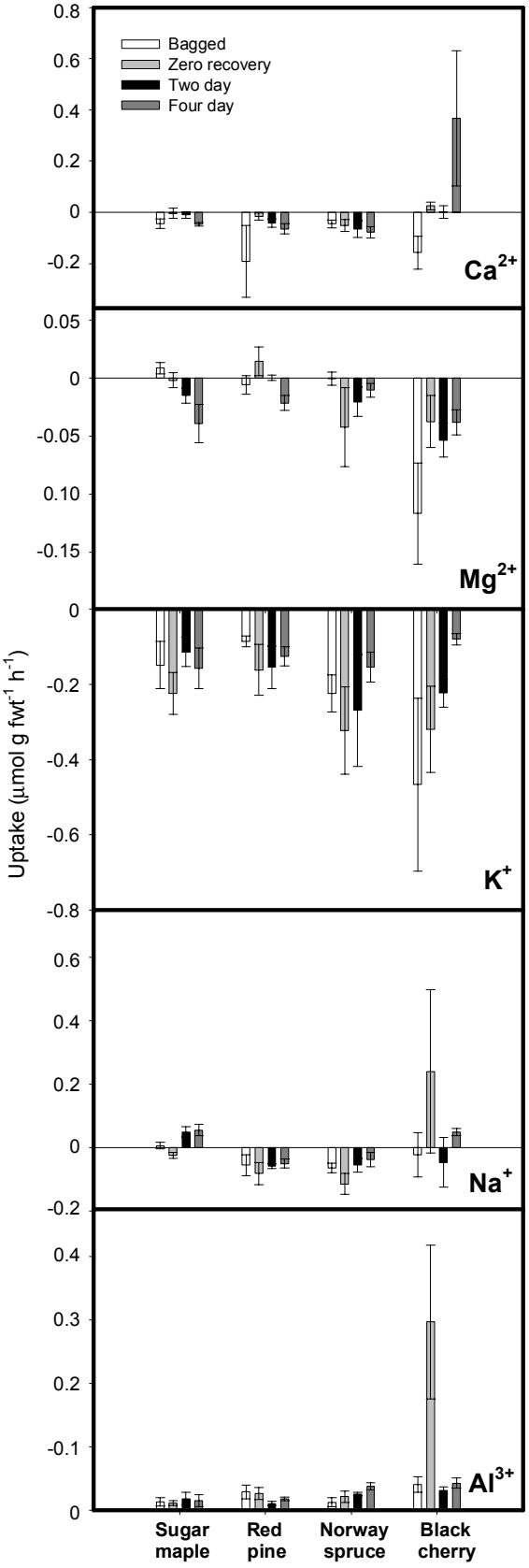
Figure 1. Mean net uptake (positive values) or net efflux (negative values) of by seven different tree species measured using the depletion method on intact roots. In 2000-2001, roots at Calhoun Experimental Forest, SC (loblolly pine), Hubbard Brook Experimental Forest, NH (beech and maple), Huntington Forest, NY (beech and maple), Loch Vale Experimental Forest, CO (spruce and fir), Fraser Experimental Forest, CO (spruce and fir) and Walker Branch, TN (chestnut and white oak) were excavated and placed in solutions that simulated soil concentrations of K^+, NH_4^+, Mg^{2+}, and Ca^{+2}. Changes in nutrient concentrations were monitored over time. Vertical bars indicate standard errors of the mean (n= 4-11).

Figure 2. Mean net uptake (positive values) or net efflux (negative values) of Ca^{+2}, Mg^{2+}, K^+, Na^+ and Al^{+3} by four tree species given pretreatments to mitigate excavation-related disturbance. “Bagged” roots were grown in bags filled with a sand-soil mixture. “Zero recovery” roots were excavated and used immediately for uptake experiments. “Two day“ and “Four day recovery“ roots were excavated and given two- or four-day recovery periods, prior to experiments. Vertical bars indicate standard errors of the mean (n=7–10). For a detailed description of the methodology and the effect of treatments on N and P uptake, see McFarlane and Yanai (2006).

Figure 3. Mean net uptake of NO_3^- and NH_4^+ by white pine seedlings grown in soil-uptake-monitoring (SUM) columns filled with sand. Uptake of NO_3^- and NH_4^+ was measured by adding KNO_3 or NH_4Cl to the SUM columns. After circulating the solution in the columns for one hour, the solution was removed by a vacuum pump and analyzed for NO_3^- or NH_4^+. Uptake rates were corrected for changes in the concentration of the controls, which did not contain plants. Vertical bars indicate standard errors of the mean (n= 3).


K+
NH4+
Mg2+
Ca2+
Uptake (µmol g fwt-1 h-1)
0.0
-0.5
-1.0
-1.5
-2.0
0.2
0.0
-0.2
-0.4
0.4
0.2
0.0
-0.2
1.2
0.8
0.4
0.0
-0.4
Loblolly pine
American beech
Sugar maple
Engelmann spruce
Subalpine fir
Chestnut oak
White oak

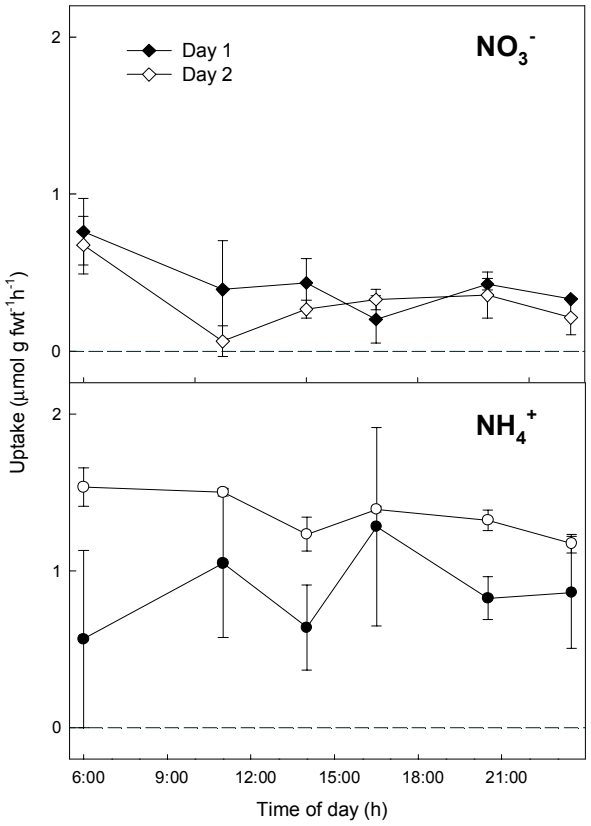
1


Bagged
Zero recovery
Two day
Four day
Ca2+
Mg2+
K+
Na+
Al3+
Uptake (µmol g fwt-1 h-1)
Sugar maple
Red pine
Norway spruce
Black cherry

1

1

2


Day 1
Day 2
NO_3^-
NH_4^+
Uptake (µmol g fwt^{-1}h^{-1})
Time of day (h)
0
1
2
6:00
9:00
12:00
15:00
18:00
21:00

3