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REVIEW

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

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Abstract Nutrient uptake by roots of mature trees 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 roots, isotopic tracers, autoradiography, depletion, and lysimeters. Although these methods have provided many insights, 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. Microbes and mycorrhizas can also affect estimates of uptake. A greater focus on methods development is critical to advancing our understanding of nutrient uptake of mature trees under conditions representative of those in the field.

Keywords Efflux · Excision · Ion uptake · Nutrient concentration · Mycorrhizas

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Introduction

Surprisingly little is known about rates of nutrient uptake by tree roots in the field, despite their importance for tree growth and survival. Root systems of trees are extensive, spatially variable, morphologically and physiologically heterogeneous, often associated with microbes and mycorrhizal fungi and frequently 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, but 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 and uptake capacity as specific root uptake at non-limiting concentrations. Other parameters are also important for estimating uptake of trees in the field, such as absorptive root surface (Van Rees et al. 1990), buffering capacity (Van Rees et al. 1990) and soil supply of nutrients (Rengel 1993), but are not addressed in this review.

In the past, specific root uptake of trees has been measured primarily using the fine roots of seedlings in solution culture. Results of these studies are difficult to extrapolate to mature trees in 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, roots of seedlings in solution culture are seldom mycorrhizal (Van den Driessche 1971; Ingestad and Lund 1979; Bledsoe and Rains 1981), whereas many trees depend on mycorrhizas to supplement nutrient uptake (Smith and Read 1997). Finally, there is little reason to expect that parameters measured on seedlings will accurately reflect

uptake by mature trees, since trees change physiologically as they age (Espeleta and Eissenstat 1998; Law et al. 2001). Despite the drawbacks, scientists still commonly use the solution culture method because rates of uptake can be readily estimated.

Current techniques for measuring specific root uptake of mature trees, the focus of this review, include excised roots, isotopic tracers, autoradiography, depletion, and lysimeters. These techniques have provided valuable information about uptake rates of trees but 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. Finally, many tree roots depend on mycorrhizas to supplement nutrient uptake (Smith and Read 1997), but few studies have quantified uptake by mycorrhizal roots of mature trees.

Continued development of methods is critical to obtain more realistic estimates of specific root uptake and improving uptake models that scale from the root to the tree. Better techniques would also improve our understanding of how nutrient uptake of trees varies among species, through the growing season, at different stages of plant development, and under different soil conditions.

In this review, we will first describe methods currently used to measure specific uptake rates of roots of mature trees and review their relative strengths and weaknesses. We classify these methods into two categories: (1) methods in which uptake of a tracer into the root or shoot tissue is measured and (2) depletion methods in which uptake rates are calculated from changes in solution concentrations over time. In the second section, we focus on the methodological challenges to obtain more realistic estimates of uptake. 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

Tracer methods

Excised roots

The excised root technique (Epstein et al. 1963) 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. If uptake of organic compounds is of interest, roots may be exposed to nutrient solution containing organic compounds double-labelled, such as with ^{13}C or ^{14}C and ^{15}N. 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 1960s and has proven extremely valuable for characterizing the nutritional status of trees (Bowen 1970; Jones et al. 1994; Hogberg et al. 1998) and measuring inorganic (Epstein et al. 1963; Huang et al. 1992) and organic (Price and Stevens 1989; Persson and Nasholm 2003) uptake by roots. Studies with excised roots were considered superior to studies with intact roots, because 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 intact roots are necessary to obtain realistic estimates of root respiration and uptake (Saglio and Pradet 1980; Bloom and Caldwell 1988). Excision of root tissue may cause a greater decline in nutrients that are actively taken up and assimilated, such as NO_3^- (Bloom and Caldwell 1988), than nutrients, such as HPO_4^{2-}, that are acquired passively (Gronewald and Hanson 1982). Moreover, the excised root method is often used to estimate 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; Kreuzwieser et al. 1997), uptake must be measured at two time intervals to differentiate between influx (5 min) and net uptake (2 h; Topa and Sisak 1997; Mata et al. 2000) or the tracer method must be combined with the depletion method (Clark et al. 2000), which is described below. Therefore, influx 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 and plant age), assuming excision does not have an interactive effect on the factor of interest. The use of this method can also be limited by the special handling and disposal procedures for radioactive tracers and the high cost of stable isotope analysis.

Intact roots

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; Choi et al. 2005), injected into the soil (Caldwell et al. 1985) or supplied to individual roots using labeled nutrient solution (Gessler et al. 1998; Warren 2006; Warren and Adams 2007). In these 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; Warren 2006), 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 (in contrast to net uptake which includes efflux) of inorganic and organic compounds 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), species preferences for NO_3^- or NH_4^+ (Choi et al. 2005; Warren 2006; Warren and Adams 2007), 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.

A new technique for determining which roots are actively taking up nutrients using isotopic tracers 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. 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. This technique might be feasible with roots of large trees using ingrowth into root bags (Comas and Eissenstat 2004).

Depletion methods

Intact roots in solution

The depletion method offers a possible improvement over the excised root method in that isotopes are not required and the roots remain intact. In this method, roots of mature trees (Gessler et al. 1998, 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. 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.

The main disadvantage to the depletion method is that the roots are excavated and the extramatrical hyphae of their fungal associates are severed, which can affect rates of uptake. In some field studies with mature trees, efflux was greater than influx (Lucash et al. 2005), although net uptake is clearly not negative over the lifetime of the plant. In the "Methodological challenges", these problems are discussed in more detail.

Intact roots in porous media

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 inoculated with mycorrhizal fungi (Colpaert et al. 1999). Nutrient solution is added to the medium and removed for sampling using a vacuum 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.

The main advantage to the lysimeter technique is that it allows specific uptake rates to be estimated without excavating the roots as with tracer studies (Colpaert et al. 1999; Scholberg et al. 2001; Lucash 2005). Another advantage 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).

There are several drawbacks to lysimeter methods. First, plants must be grown in perlite or sand to minimize adsorption of nutrients on soil surfaces. Unless kept sterile, the perlite or sand would contain microbes which also take up nutrients. This would complicate interpretation of uptake rates as discussed below. Second, the nutrient solution needs to be mixed and aerated to control the concentration of nutrients at the root surface and to prevent root anoxia (Escamilla and Comerford 1998). Third, the mass of roots is typically assumed to remain constant throughout the period of uptake, which may not be true during periods of rapid root growth. Finally, the lysimeter technique cannot be used for large trees, since the entire root system is limited by the size of the container.

Methodological challenges

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

Bias introduced by root sampling approaches

Selecting which roots to sample and when to sample are 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 lateral 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 2-year study, uptake of phosphate by excised roots of balsam fir saplings 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 mature spruce was similar in the first and second years, but uptake by intact beech roots was significantly higher in July and September 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). Short-term experiments may also overestimate uptake rates, since adsorption on the surface and loading in the Donnan free space of roots occurs primarily in the first few minutes of exposure to nutrient solution (Kronzucker et al. 1995). 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 (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). Microelectrodes can be used to sample solutions on a continuous basis (McClure et al. 1990; Kochian et al. 1992) but accurate probes are difficult to construct and generally too fragile for use in soil.

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, they 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; 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, 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.

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 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 a depletion study with mature trees, roots of four tree species were exposed to pretreatments designed to reduce the effects of disturbance on uptake measurements (McFarlane and Yanai 2006). Roots were either excavated directly from the soil, excavated and allowed to recover for 2 or 4 days, or grown in bags containing a sand–soil mixture to reduce damage to the roots. Unexpectedly, roots with less root damage and those allowed a recovery period did not have consistently higher uptake rates than roots recently excavated (Fig. 1),

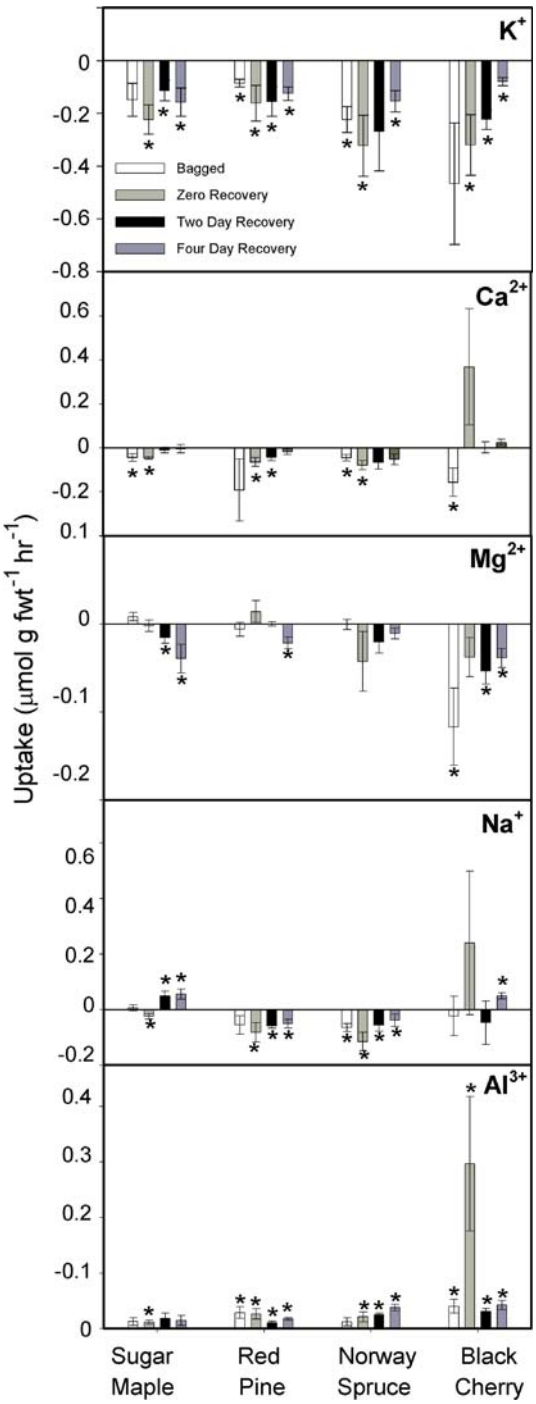

Fig. 1 Mean net uptake (*positive values*) or net efflux (*negative values*) of Ca^{+2}, Mg^{2+}, K^+, Na^+ and Al^{+3} by four tree species given pretreatments intended to mitigate excavation-related disturbance. "Bagged" roots were grown in bags filled with a sand–soil mixture. "Zero recovery" roots 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. *Asterisks* indicate that means were significantly different from zero at $\alpha = 0.05$. *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)

indicating that either disturbance was not important in this study or that the pretreatments designed to minimize disturbance were not 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 2005). 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 study is needed 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 typically detects nutrient influx but not efflux. In some situations, the rate of efflux can be a significant component of net uptake. A few studies have examined both influx and net uptake using isotopic tracers, either by use of double isotopes (Kreuzwieser et al. 1997) or by short durations (5 min) to estimate influx and longer durations (2 h) for net uptake (Topa and Sisak 1997; Mata et al. 2000). In these studies, efflux rates were 78% of gross NO_3^- influx in American beech (Kreuzwieser et al. 1997), 63–69% of NO_3^- influx in cork-oak (Mata et al. 2000) and 45–79% of P influx in loblolly pine (Topa and Sisak 1997) seedlings grown in hydroponics. Efflux rates exceeded influx rates for Ca^{+2} and K^+ in Sitka spruce, Douglas-fir and western hemlock seedlings in hydroponics (Rygiewicz et al. 1984). In a set of studies using the depletion method with mature trees, we found that net uptake of NH_4^+, $Mg^{2+}$and Ca^{+2} over a 2-h period was positive for some species but not others; net uptake of K^+ was consistently negative (Fig. 2).

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. Even in studies where net uptake is positive, it is difficult to determine if the efflux rates are representative of rates in the field, since estimates are highly dependent on experimental conditions, such as the timing of sampling (Scheurwater et al. 2000), the nutritional status of the plant (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). The importance of measuring nutrient efflux has not been clearly recognized, perhaps because tracer methods often estimate only gross influx. The need remains for methods that

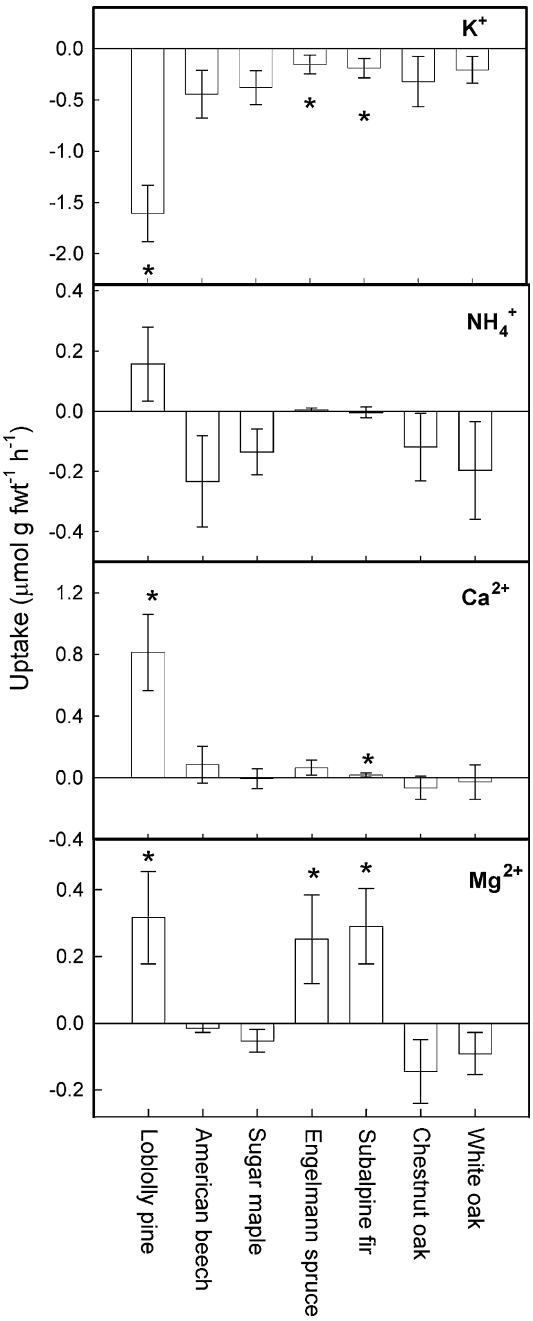

Fig. 2 Mean net uptake (*positive values*) or net efflux (*negative values*) by seven different tree species measured using the depletion method on intact roots. In 2000–2001, roots at Calhoun Experimental Forest, SC (loblolly pine), Huntington Forest, NY (beech and maple), Loch Vale Experimental Forest, 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. *Asterisks* indicate that means were significantly different from zero at $\alpha = 0.05$. Vertical bars indicate standard errors of the mean (n = 4–14)

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 seedlings and mature trees (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), which is an improvement over arbitrary or unrealistically high solution concentrations. An even better approach is to estimate concentrations at the root surface, but these estimates rely on destructive methods (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, such as micro suction cups (Dieffenbach et al. 1997; Dieffenbach and Matzner 2000) and biosensors (Jaeger et al. 1999), 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:

$$U = \frac{V_{\max}(C_0 - C\,\min)}{K\mathrm{m} + (C_0 - C\,\min)}$$

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. Also, net uptake rates of trees are often fitted to the Michaelis–Menton equation, even though the model was developed using only unidirectional fluxes (Price and Stevens 1989; Gessler et al. 2005). Depending on the concentrations used in the study, uptake may be linearly related to concentration, as observed for P uptake in red maple seedlings (Kelly and Kelly 2001) and NO_3^- uptake in mature 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;

Table 1 Ammonium concentrations used to quantify uptake capacity in nine studies involving tree species, in order of increasing concentration

Species	Conc. (μmol l^{-1})	Technique	Author
Fagus sylvatica	53–55	Depletion	Gessler et al. (1998)
Picea abies			
Picea abies	0–150	Depletion	Marschner et al. (1991)
Acer rubrum	0–200	Depletion	BassiriRad et al. (1999)
Acer saccharum			
Pinus ponderosa	0–500	Excised	BassiriRad et al. (1997)
Pinus taeda			
Populus tremuloides	0–500	Excised	Rothstein et al. (2000)
Picea abies	800	Lysimeter	Eltrop and Marschner (1996)
Acer saccharum	0–1,000	Excised	Rothstein et al. (1996)
Betula alleghaniensis			
Carya ovata			
Fagus sylvatica			
Fraxinus americana	0–4,000	Excised	Lajtha (1994)
Liriodendron tulipifera			
Prunus serotina			
Quercus phellos			

Siddiqi et al. 1990). In one study, uptake rates of NH_4^+ by Douglas-fir seedlings 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).

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). Nutrient uptake during the experiment can also change nutrient concentrations and ratios. Consideration should be given to selecting the concentrations and nutrient combinations for uptake experiments.

Influences of microbes and mycorrhizas on estimates of uptake

Most studies assume that microbial uptake rates at the root surface are negligible. However, microorganisms are found on root surfaces at high densities, even in plants grown in solution culture. Microbial uptake can lead to interpretive errors, since microbes can cause changes in nutrient availability through processes such as nitrification. Microbial uptake is sometimes estimated from root-free controls, but this approach underestimates microbial activity, since microbial biomass is higher in the presence of roots. Antibiotics have been used to reduce bacterial populations on the root surface but these treatments have not been shown to be effective (Smart et al. 1995). That study also concluded that nitrification on root surfaces of wheat in hydroponics is negligible compared to root uptake of NH_4^+, but the rate for tree roots grown in the field is unknown. New methods are necessary to separate uptake by roots and the microbes on their surface.

Most trees in the field are associated with mycorrhizal fungi, but no studies to date have studied the effects of mycorrhizas on uptake by mature trees. Instead, uptake by mycorrhizal roots has been studied using tree seedlings in solution culture. Seedlings are grown in soil to allow mycorrhizal development and then transferred to hydroponic solution for uptake measurements (Rygiewicz et al. 1984; Cumming 1996; Constable et al. 2001; Wallander et al. 1997). These studies indicate that mycorrhizas are important for uptake of Ca^+ and Mg^{2+} but not PO_4^{-3} or K^+ in Scots pine seedlings (Boxman and Roelofs 1987). Mycorrhizas have been shown to stimulate NO_3^- uptake in maritime pine (Plassard et al. 1994), Norway spruce (Marschner et al. 1991), Scots pine (Wallander et al. 1997) and loblolly pine (Constable et al. 2001) but the effects of mycorrhizas on NH_4^+ uptake vary with species. Mycorrhizal infection increased NH_4^+ uptake by roots of Norway spruce (Marschner et al. 1991) but not loblolly pine (Constable et al. 2001).

Using seedlings excavated from soil to study mycorrhizas is problematic, because the extramatrical hyphae of mycorrhizae are severed during excavation. Only one study to date has measured uptake by mycorrhizal tree seedlings in intact soil. In that study, NO_3^- uptake was similar in mycorrhizal and non-mycorrhizal Norway spruce seedlings (Colpaert et al. 1999).

Two methods, fungicide treatments and mesh bags, could be used to measure uptake by mycorrhizal seedlings and mature trees. Fungicide treatments have been shown to eliminate vesicular-arbuscular mycorrhizal colonization and reduce P uptake in peas (Jakobsen and Nielsen 1983; Schweiger and Jakobsen 2000) and corn (Lu and Miller 1989). This method gives a rough estimate of the fungal contribution to uptake in the field but the fungicide eliminates other soil organisms, which could affect nutrient uptake. The mesh bag method uses a more direct approach to measuring hyphal uptake by placing isotopically labeled soil in mesh bags in the field and allowing fungal hyphae to grow into the bags and take up nutrients (Schweiger and Jakobsen 1999; Jakobsen et al. 2001). Most studies using this method, however, quantify uptake by fungal hyphae but not roots. Mesh screens of different diameters were used to compare uptake among mycorrhizal roots, hyphae, and bulk soil using mesh screens of different diameters but that study was conducted in the greenhouse and not in the field (Cheng and Baumgartner 2006). The mesh bag method holds promise for obtaining more realistic estimates of uptake by AM and ectomycorrhizal roots of seedlings and mature trees.

Conclusions

Our limited ability to assess specific nutrient uptake by roots of mature trees restricts our understanding of a key process associated with tree function and the factors that affect it. The most widely used method to measure uptake relies on tree seedlings in solution culture. Tracer and depletion methods have been used to measure uptake by intact roots of mature trees but these techniques depend upon observations from roots that have been disturbed or grown under artificial soil conditions. Although these

estimates of uptake may be useful for comparative purposes, more realistic estimates are necessary to describe root uptake by mature trees in the field.

The task of measuring specific root uptake by trees is limited by methodological problems which need to be addressed. No method is without some shortcomings, and researchers need to carefully evaluate the strengths and weaknesses of various methods for their specific purposes.

Although considerable progress has been made in the measurement of nutrient uptake by trees, many questions remain unanswered about how and when tree roots take up nutrients. 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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