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Estimating nitrogen uptake of individual roots in container- and field-grown plants using a ^{15}N-depletion approach

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Abstract. We only have a limited understanding of the nutrient uptake physiology of individual roots as they age. Despite this shortcoming, the importance of nutrient uptake processes to our understanding of plant nutrition and nutrient cycling cannot be underestimated. In this study, we used a ^{15}N depletion method that allowed for the measurement of nitrate-N uptake rates on intact, individual, fine roots of known age. We expected that N uptake would decline rapidly as fine roots aged, regardless of the environmental conditions and species used. We compared age dependent uptake patterns of young grape cuttings with those of mature vines and with those of tomato. Although patterns of declining uptake with increasing root age were similar for all species and conditions tested, large differences in maximum N uptake rates existed between young cuttings and mature vines, and between woody and herbaceous species. Maximum rates were 10-fold higher for tomato and 3-fold higher for the grape cuttings, when compared with uptake rates of fine roots of mature vines. Coefficients of variation ranged from 43 to 122% within root age groups. The large variability in physiological characteristics of fine roots of the same age, diameter and order suggests that there is a functional diversity within fine roots that is still poorly understood.

Additional keywords: fine roots, nitrate uptake, nutrient uptake, root age, root diameter, root function.

Introduction

The physiology of plant nutrient acquisition under field conditions is important to both agriculture and ecology. Competition below ground for limited nutrients is a key factor influencing the success of species (e.g. Wilson 1988; Robinson *et al*. 1999), and the ability to acquire and assimilate nutrients is a key determinant of net primary production (Marschner *et al*. 1986). The ability of plants to acquire nutrients is determined by the amount of root absorptive surface area, soil conditions, and specific rates of root uptake. Understanding factors that influence the uptake of nutrients at the root surface requires a greater understanding of the traits of individual roots, as root physiology, anatomy and morphology can change dramatically depending on order, age and symbiotic association.

Many studies have focussed on the influx and efflux of water and nutrients along single root axes (Russell and Sanderson 1967; Clarkson *et al*. 1968; Eshel and Waisel 1972, 1973; Ferguson and Clarkson 1975; Clarkson and Scattergood 1982; Henriksen *et al*. 1990; Siddiq *et al*. 1991; Wang *et al*. 1993; Kronzucker *et al*. 1995) or whole-root systems of young plants grown in hydroponic systems (Bloom 1985; Smart and Bloom 1988; Le Bot and Kirkby 1992; Raper *et al*. 1991; Andriolo *et al*. 1996). Studies performed along individual root axes generally showed that the active zone of nutrient uptake is the zone directly behind the root tip. This was interpreted as younger root tissue being more active in nutrient uptake than older root tissue, with the older root tissue located further back along the root axis. However, many absorptive roots, especially in woody plants, only extend 2 or 3 cm in the first days of life and then stop growing (e.g. Resendes *et al*. 2008). These short,very fine lateral roots age, but we have a limited understanding of their nutrient uptake physiology as they age (Lucash *et al*. 2007), particularly in light of the fact

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the tip ceases growing (using an ordering scheme where roots with no laterals are 1st order) (Fitter 1982).

Such fine lateral roots are thought to be important to nutrient acquisition under field conditions. This is underscored by whole-root system studies which show that the most absorptive roots may represent only a small fraction of the total root system mass. For example, in a study comparing apparent inflow rates (using plant growth and tissue N content) and actual (measured) inflow rates, Robinson *et al*. (1991) concluded that no more than 10% of the root system of spring wheat was required for the plants' acquisition of nitrate from the soil. Similarly, Passioura (1980) estimated that the fraction of the wheat root system active in water uptake was no higher than 30%.

Others have described the root system in the context of branching orders (Macfall *et al*. 1991; Guo *et al*. 2004; Eissenstat and Volder 2005; Guo *et al*. 2008), with the most external, 1st and 2nd order roots mainly responsible for the acquisition of mineral nutrients and water, while the older, higher-order roots (e.g. >4th order) provide support, transport and anchoring as well as storage (Guo *et al*. 2008; Valenzuela-Estrada *et al*. 2009). However, 1st and 2nd order roots are themselves variable populations. A single plant will have a large number of 1st and 2nd order roots that vary widely in age (Bouma *et al*. 2001; Volder *et al*. 2005). The young cohort of 1st order roots (typically <25 mm in length) is considered the most important portion of the root system for N acquisition (Jensen 1962; Colmer and Bloom 1998; Taylor and Bloom 1998), and, as such, represent only a very small fraction of total root system mass or length. Often 1st order roots may only grow for a short duration (3–7 days) before elongation stops (e.g. Resendes *et al*. 2008). The ability of these 1st order roots to acquire N and phosphorus dramatically decreases with increasing age (Bouma *et al*. 2001; Volder *et al*. 2005). Volder *et al*. (2005) showed in a greenhouse study in grape (*Vitis rupestris* Scheele × *V. riparia* Michx. cv. 3309C) that roots that were only 2 days old already had a 50% reduction in N uptake rate compared to that at birth. Bouma *et al*. (2001) showed strong age-related declines for phosphorus uptake in the field in apple (*Malus domestica* Borkh.) and orange (*Citrus aurantium* L.).

It is unclear whether results based on cuttings grown under greenhouse conditions (e.g. Volder *et al*. 2005) accurately reflect uptake patterns of mature plants under field conditions. Is the rapid decline observed in root functioning of greenhouse plants the same for roots of plants growing in the field? Moreover, do herbaceous annual plants show similar patterns as those observed in woody species? Part of the reason for limited research in the area is methodological. Many field techniques measuring N uptake rates have proved problematic for reasons not entirely clear (Lucash *et al*. 2005, 2007). In this study, we used a ^{15}N depletion method that allows for the measurement of uptake rates on intact, individual fine roots of known age to compare age-dependent effects of ^{15}N uptake in grape under greenhouse and field conditions. We also explored how N uptake in an annual herbaceous species (tomato) compares with that of a woody species (grape). We hypothesised that roots of seedling grapes in the greenhouse would exhibit higher N uptake rates than those of mature woody plants in the field. We also hypothesised that N uptake of individual roots of a fast-growing herbaceous plant like tomato would be higher than that observed in individual roots of grape seedlings. In all experimental systems, we predicted strong declines in uptake with root age.

Materials and methods

Tomato experiments

Tomato (*Lycopersicon esculentum* L., cv. 174) cuttings were grown in 3.8-L pots filled with Sunshine potting mix (Sun Gro Horticulture Canada Ltd, Vancouver, Canada), either in growth chambers (400 μmol m^{-2} s^{-1} PPFD, 70% RH, 25°C) at University of California, Davis (exp. 1), or in a glass greenhouse at Penn State University (exp. 2). 'Windows' of ~20 × 15 cm (w × h) were cut into the pots and covered with transparent, thin, acetate film. The windows were then covered with light impenetrable shade cloth. New root growth was traced on the windows daily, with different coloured permanent markers. Root growth in the windows was generally observed within 10 days after planting. After 3 weeks of root growth, an incision was made in the acetate, and a root of known-age was removed from the substrate, but kept attatched to the plant. Care was taken to avoid roots that carried laterals or roots where there were doubts about root age. Roots were placed in 0.7-mL (exp. 1) or 0.4-mL (exp. 2) eppendorf vials that were filled with buffer (10 mM MES, 1 M $CaSO_4$, 5 μM K_2HPO_4 at pH = 5.7) and 1 mM unlabelled KNO_3. Vials were covered with parafilm and the window was covered with shade cloth. Roots were left to adjust to their new conditions for at least 5 h.

Following the period of adjustment, each root was gently washed with nitrogen-free buffer, and blotted dry. Organic specks were carefully removed using fine-pointed forceps. Intact 1–2 cm long roots with root tips were individually inserted into a new vial filled with a known volume of buffer and 1 mM (exp. 1) or 0.1 mM (exp. 2) $K^{15}NO_3$. Thus, mainly the portion of the root that is active in N uptake was examined (Colmer and Bloom 1998; Taylor and Bloom 1998). The vials plus intact root tips were then gently taped to the window and loosely covered with parafilm to prevent evaporation, while allowing oxygen exchange with the air to prevent development of anaerobic conditions. The procedure was repeated with other roots in the window (~5 roots per plant, six plants total) and a vial with buffer without a root was also taped to the window of each plant as a control. The light-impenetrable cloth was placed back over the window. After the roots had been in the vial for 1 h, 10-μL samples were taken from the solutions and pipetted onto filter paper discs in a 96-well microplate. The filter paper discs were previously treated with 5 μmol of background N [10 μL 0.25 M $(NH_4)_2SO_4$] and allowed to dry. The microplate was kept covered until the samples were pipetted on the filter paper disc and then closed immediately. Samples taken from the uptake solution were pipetted onto new discs after 2, 4 and 6 h of root insertion, as well as five samples from the initial solution at $t = 0$ h. Roots were harvested after 5 h by cutting them at the edge of the vial and rinsing them in distilled water. At the end of the

uptake period, the vials were closed immediately and the volume of the remaining solution was determined by weighing. Roots were scanned on a flatbed scanner with a transparency adaptor (WinRhizo, Regent Systems, Quebec City, Canada) and analysed for colour, length and diameter. After scanning, roots were placed in a forced air oven at 65°C and left to dry until stable mass. Root mass was determined on a microbalance; only roots with masses >0.1 mg were used for further analyses.

The paper discs were analysed for ^{15}N at the Stable Isotope Research facility for Environmental Research (SIRFER) at UC Davis. After correcting for background enrichment (unused filter discs with 5 µmol N) and volume changes in the vials, the decrease in ^{15}N content of the vial ($^{15}N_{t0} - {}^{15}N_{t1}$) was calculated at each time point. The total decrease in ^{15}N per vial was then divided by the root mass and the time expired between the two measurement points to express uptake on a per mass per unit time basis.

Uptake = $(^{15}N_{t_0} - {}^{15}N_{t_1})/[(t_1 - t_0) \times$ root mass], where $^{15}N_{t0}$ is the total amount of ^{15}N (nmol) in the vial at the start of the measurement period (t_0) and $^{15}N_{t_1}$ is the total amount of ^{15}N left in the vial at the end of the measurement period (t_1), $t_1 - t_0$ is the length of the measurement period in seconds, and root mass is the dry mass of the portion of the root that was in contact with the solution (mg).

In addition to vials with roots, control vials without roots were also placed on the windows and any changes in ^{15}N content were measured. The total uptake rate per vial with a root ($^{15}N_{t_0} - {}^{15}N_{t_1}$) was corrected for any changes observed in the control vials over the same time period (for more information, see Volder *et al*. 2005). For most roots (except very old ones), the depletion of ^{15}N caused by root uptake greatly exceeded the average change in the control vials. For each root we also measured root length and we divided mass based uptake rates by the specific root length ($m\,g^{-1}$) to express uptake rates on a per length basis. Final solutions from exp. 1 were also analysed on a HPLC with UV detector for total NO_3^- content. This allowed for a comparison of uptake rates measured via HPLC and via the ^{15}N method (Fig. 1). These results show that uptake as measured via HPLC or using changes in $^{15}N/^{14}N$ ratio were closely correlated.

Concord grape experiments

Seedlings of grape (*Vitis labruscana* Bailey cv. Concord) were transplanted into 3.7-L pots filled with Sunshine mix (Sun Gro Horticulture Canada Ltd, Vancouver, CA, USA) and grown in the greenhouses of the Department of Horticulture at the Pennsylvania State University. The seedlings were planted in April 2000 and uptake measurements were conducted in June 2000. Root growth and nitrate-N uptake rates of individual intact fine roots were measured as described above.

For the field study, root observation boxes were placed in the root zones of mature Concord vines in April 2000 at Cornell University's Vineyard Laboratory in Fredonia, NY, USA. The vines were growing in soils that were a very deep (>3 m), very well drained, Chenango gravelly loam that was relatively uniform across the plot. Study plants were mature,

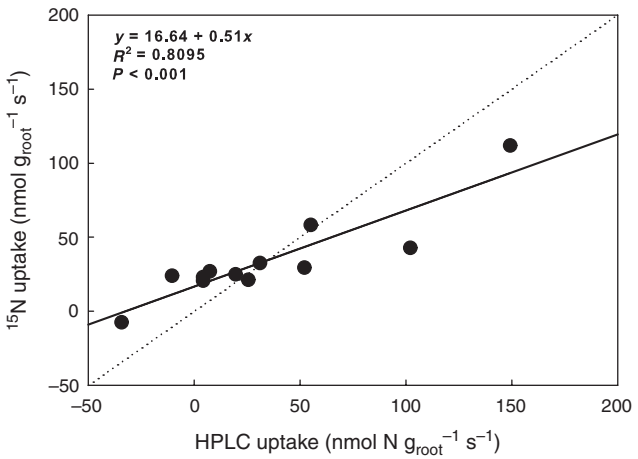

Fig. 1. Relationship between N uptake as measured via HPLC and the ^{15}N method on the same intact fine tomato roots (1 mM N initial solution). The dotted line represents a 1 : 1 line.

25-year-old *V. labruscana* cv. Concord grapevines with permanent cordons 1.8 m above the ground and spaced at 2.4 m between vines and 2.7 m between rows as described by Anderson *et al*. (2003). Roots were traced for 12 weeks until August 24 and 25 and measurements of N uptake were performed using the technique described above.

Statistics

Relationships between the two uptake methods and between vial N concentration and time were fitted using linear regression and slopes and intercepts were tested for significant deviations from 0 using the graphics software Sigmaplot 9.0 (Systat Software Inc., San Jose, CA, USA) and the JMP 7.01 statistical package (SAS Institute, Cary, NC, USA). Best fit curves for the relationships between root age, N uptake, and root diameter were determined using the graphics software Sigmaplot 9.0 (Systat Software Inc.) and tested for statistical significance using the JMP 7.01 statistical package (SAS Institute).

Results

The amount of ^{15}N in the vials generally declined linearly over time, demonstrating a constant rate of ^{15}N uptake over the measurement period (Fig. 2), suggesting that concentrations of oxygen and nitrate in the vial remained high enough to support nitrate uptake. When this decline was calculated as amount of ^{15}N taken up per gram dry root per second (Fig. 3), we found a rapid decline in ^{15}N uptake rate with increasing root age for tomato fine roots. Uptake rates had declined by 50% after 8 days. The rapid decline in uptake rate was significant, both when expressed on a mass or on a length basis (data not shown). We found a similar relationship between root age and N uptake rate for fine roots of greenhouse-grown Concord grape seedlings (Fig. 4, inset) and fine roots of mature Concord grape vines (Fig. 4). Maximum rates of uptake were lower for the mature grape vines than for the greenhouse-grown grape seedlings (Fig. 4). Maximum uptake rates for the tomato

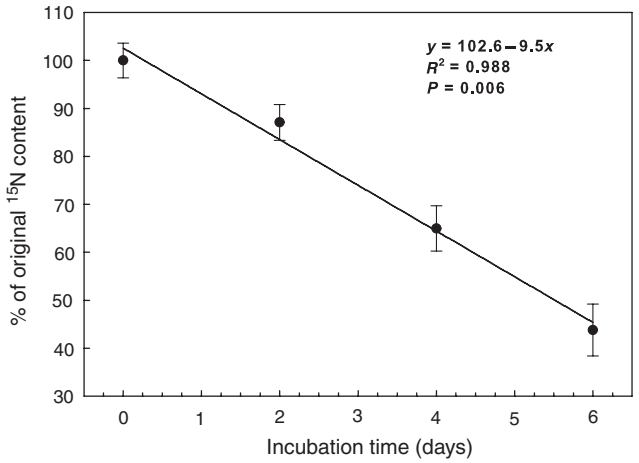

Fig. 2. The influence of root incubation time on ^{15}N (% of original) remaining in the vials (0.4 mL) after correcting for volume changes using intact fine tomato roots (0.1 mM nitrate-N initial concentration; $n = 27$, ± s.e.). Slopes of individual lines were used to estimate uptake rates.

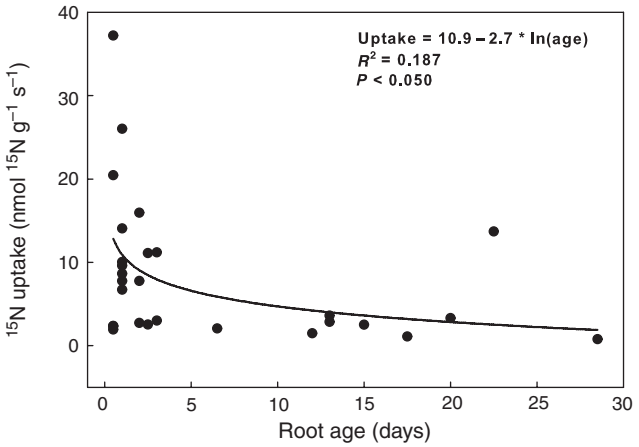

Fig. 3. The relationship of N uptake with root age in intact tomato roots.

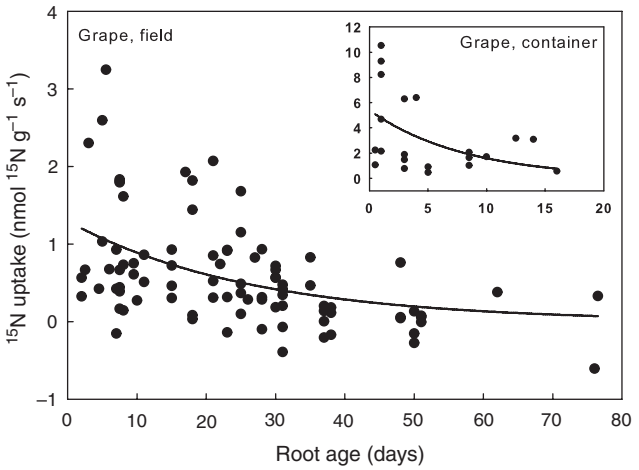

Fig. 4. The relationship of ^{15}N uptake with root age for field-grown and container-grown (inset) individual Concord grape fine roots. Note the difference in scales between the field- and container-grown dataset. The relationship for the field grown roots was: uptake = 1.30 × exp(−0.038 × root age), $R^2 = 0.233$, $P < 0.001$. The relationship for the container-grown roots was: uptake = 5.41 × exp(−0.072 × root age), $R^2 = 0.194$, $P < 0.050$.

fine roots were an order of magnitude higher than those of fine roots of field grown, mature, Concord grape (Figs 3, 4). Within species and root age classes a wide range of uptake rates was found and coefficients of variation ranged from 43 to 122% (Table 1).

There was no relationship between ^{15}N uptake and fine root diameter for either the container- or field-grown grape roots ($P > 0.24$, data not shown), but both mass-based and surface-area-based ^{15}N uptake rates of fine tomato roots declined with increasing diameter (Fig. 5). A possible underlying reason for the inverse relationship of ^{15}N uptake and diameter in tomato is that coarser roots might be older; however, we observed no relationship between root diameter and root age in tomato (Fig. 5*B*, inset), nor was there such a relationship for the 1st order grape roots (data not shown).

Table 1. Average ^{15}N uptake rates for several root age classes in this and earlier investigations
Average rates are mean ± s.d.; CV, coefficient of variation

	n	Age range (days)	Average rate (nmol $^{15}N\,g_{dw}^{-1}\,s^{-1}$)	Range (nmol $^{15}N\,g_{dw}^{-1}\,s^{-1}$)	CV (%)	Citation
			Greenhouse-grown cuttings			
Vitis rupestris × *V. riparia* cv. 3309C	3	0–1	10.05 ± 5.08	4.68–14.79	51	Volder *et al.* (2005)
Vitis rupestris × *V. riparia* cv. 3309C	10	11–23	3.22 ± 1.38	1.15–5.61	43	Volder *et al.* (2005)
Vitis labruscana cv. Concord	14	0–5	4.03 ± 3.49	0.47–10.54	87	This investigation
Vitis labruscana cv. Concord	7	9–16	1.90 ± 0.98	0.57–3.18	51	This investigation
			Mature field vine			
Vitis labruscana cv. Concord	25	2–10	0.97 ± 0.85	–0.15–3.25	88	This investigation
Vitis labruscana cv. Concord	64	11–77	0.44 ± 0.54	–0.60–2.07	122	This investigation
			Greenhouse-grown tomato			
Lycopersicon esculentum	19	0–3	10.60 ± 9.18	1.92–37.20	87	This investigation
Lycopersicon esculentum	9	7–29	3.49 ± 3.95	0.77–13.70	113	This investigation

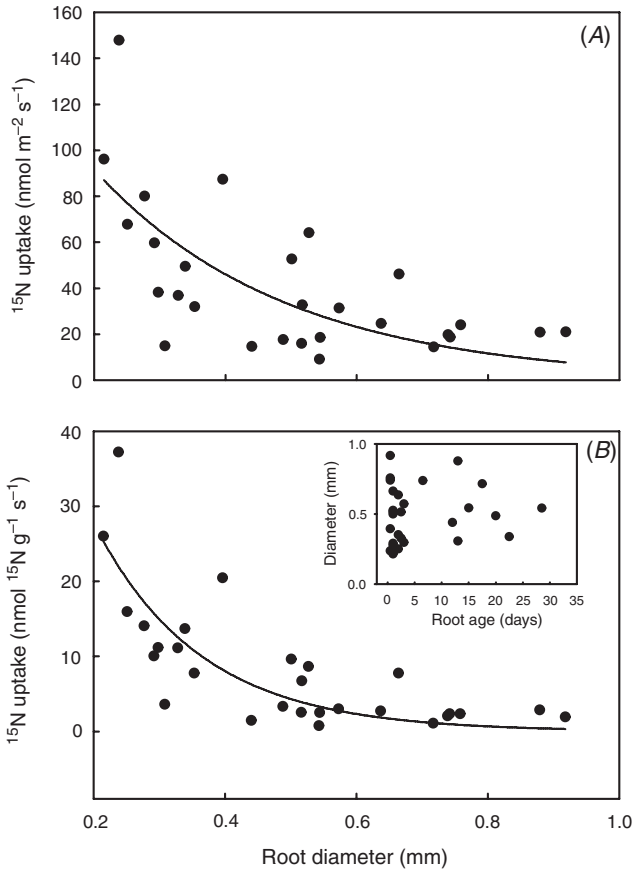

Fig. 5. The relationship of ^{15}N uptake (*A*) per unit root surface area, and (*B*) per unit dry mass with root diameter for tomato roots. The regression line in (*A*) is: uptake = 182 × exp(−3.44 × root diameter), $R^2 = 0.448$, $P < 0.001$. The regression line in (*B*) is: uptake = 96.2 × exp(−6.2 × diameter), $R^2 = 0.624$, $P < 0.001$. The inset in (*B*) shows the lack of a relationship between root age and root diameter for tomato roots.

Discussion

Root age and nitrate uptake

In this study, we have shown that both root diameter and root age can have a large effect on the nitrate-N uptake capacity of individual roots. Both roots of young cuttings and mature vines of a woody species (grape, *V. labruscana* cv. Concord) and roots of an herbaceous species (tomato, *L. esculentum* cv. T5) showed the same general pattern: a rapid decline in nitrate-N uptake capacity as roots age. This same pattern also emerged for grape cuttings of *V. riparia* × *V. rupestris* cv. 3309C in a previous greenhouse experiment (Volder *et al*. 2005). Maximum rates reported, however, were 10-fold higher for the herbaceous species and 3-fold higher for the seedlings, compared with uptake rates of fine roots of mature vines growing in the field. Thus, although the patterns were the same, large differences in maximum uptake existed between young cuttings and mature trees, and between woody and herbaceous species. These differences are likely related to plant growth rate and N demand (Poorter *et al*. 1990; Ter Steege *et al*. 1999), with faster growing plants such as seedlings or herbaceous species having higher specific N uptake rates.

We found a large variability in rates of nitrate-N uptake within fine roots of the same order and age range (Table 1). There is a sizable portion of fine roots that remains relatively inactive even from the first days of birth. Other age related studies, such as those on P uptake and respiration (Bouma *et al*. 2001), root browning (Comas *et al*. 2000), N uptake and respiration (Volder *et al*. 2005), and fungal infection rate (Resendes *et al*. 2008) found similar variation in root function within fine roots of the same age, where a large proportion of the roots appears to be inactive.

Some roots, commonly called ‘pioneer’ or ‘framework’ roots that often become part of the woody root system infrastructure, may be less physiologically active in nutrient absorption and less prone to mycorrhizal colonisation than roots that are destined to remain 1st order roots (Wells and Eissenstat 2003). These pioneer roots often have a larger root tip, are born with a larger diameter, and extend in a more indeterminate fashion than those roots that are likely to remain only 1st or 2nd order and never develop secondary growth (Eissenstat and Achor 1999). Among the 1st order roots that are very fine and never undergo secondary growth, there may be distinct differences in function. For example, in apple, some of the very fine, 1st order roots are colonised within the first week by mycorrhizal fungi and have slightly higher growth rates than other similarly fine roots (Resendes *et al*. 2008). The latter may not be growing quite as actively for the first 3 days of life and soon (within 2 weeks) become colonised by non-mycorrhizal fungi or never become colonised. Thus, although new roots may be 1st order and of the same age, they may not all be destined to have the same function.

A large amount of variation in nutrient uptake rates by mature trees estimated *in situ* can be explained by the wide variety of methods employed (Lucash *et al*. 2007). Current methods, including the method employed in this paper, often do not account for diurnal variation in uptake (Scheurwater *et al*. 1999), and commonly lack adequate temperature control. However, even when the same method is employed for a short period, rates measured can vary widely (Gessler *et al*. 1998; Lucash *et al*. 2005). Nitrogen uptake rates have been reported to vary seasonally (Gessler *et al*. 1998), diurnally (Hansen 1980; Raper *et al*. 1991; Le Bot and Kirkby 1992; Cardenas-Navarro *et al*. 1998; Peuke and Jeschke 1998) and spatially along the root axis (Russell and Sanderson 1967; Henriksen *et al*. 1992; Colmer and Bloom 1998). Based upon our studies with grape cuttings, mature vines and an herbaceous plant, we conclude that nitrate-N uptake rates also can decline both as fine roots age and as diameter increases. This explains some of the seasonal variation that is reported in the literature, as maximum nitrate-N uptake rates of subsets of roots will be strongly influenced by the average age of the roots in that sample, even if the root diameter in the sample is controlled.

Both the production rate of new roots and median root lifespan will affect the average root age of a random sample of roots. During periods with high rates of root production, the root system will have a larger proportion of relatively

young root tips and this will be reflected in a random sample. Thus, during a period of high root production (e.g. late spring) we expect much higher uptake activities in an average root sample than during a period of reduced root growth (e.g. late summer), as was found by Lucash *et al*. (2005) for loblolly pine (*Pinus taeda*). In a subalpine area, where new root production may not start until the summer, Gessler *et al*. (1998) found higher uptake of ammonium during the summer than in the spring.

Root production and root longevity are affected by a great number of environmental factors, including species (Eissenstat and Yanai 1997; Black *et al*. 1998), season (Hendrick and Pregitzer 1993*a*), climatic conditions (Hendrick and Pregitzer 1993*b*), soil texture and structure (Hendrick and Pregitzer 1997), root depth (Wells and Eissenstat 2001; Baddeley and Watson 2005), plant age (Baddeley and Watson 2005) and root diameter and order (Wells and Eissenstat 2001, 2003; Baddeley and Watson 2005). The effect of root age does not only partially account for variation of N uptake measurements through time, but also in space. Roots usually have a longer lifespan deeper in the soil (Wells and Eissenstat 2001; Anderson *et al*. 2003; Joslin *et al*. 2006; Peek *et al*. 2006), increasing the average root age, which would suggest that measurements of nitrate-N uptake on random root samples from deeper soil layers would show reduced uptake rates compared with uptake rates measured on random samples from shallow soil layers. Bhat (1982) found a large decrease in nitrate uptake rates per unit root length for deeper growing apple roots in one of two apple cultivars studied, but not in the other. However, the roots used in the estimation of nitrate uptake rate in this study were growing in nutrient solution for the preceding 16 months, thus creating roots more typical of solution culture than of roots developed in soil under natural field conditions.

Implications for modelling N uptake

Plant-based ecosystem N uptake models would be improved by taking into account not only the total standing root length, but also the proportion of roots in the 0–10 day range when roots have the highest uptake capacity. Accurately quantifying and modelling fluxes of nutrients from plants to soil and *vice versa* remains one of the principal challenges in trying to predict impacts of environmental change on ecosystems (Norby and Jackson 2000). A combined index of new root production rate and standing root length (e.g. a weekly rate of new root length turnover) could be a better predictor of ecosystem nitrate-N uptake dynamics than standing root length alone.

Conclusion

Plants exhibit a rapid decline in nitrate uptake with root age regardless of growth conditions (greenhouse *v.* field) or whether the plant is woody or herbaceous. The magnitude of maximum uptake, however, varies with the environmental conditions, species, and growth demand of the plant. The large variability in physiological characteristics of fine roots of the same age and order suggests that there is a functional diversity within fine roots that is poorly understood. The rapid decline in maximum nitrate uptake as roots age partially explains why measurements of nitrate uptake on random root samples are generally not consistent through time and space, as these samples are generally highly variable in mean root age.

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