Rapid decline in nitrate uptake and respiration with age in fine lateral roots of grape: implications for root efficiency and competitive effectiveness

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Summary

• Changes in function as an individual root ages has important implications for understanding resource acquisition, competitive ability and optimal lifespan.
• Both nitrate uptake and respiration rates of differently aged fine roots of grape (*Vitis rupestris* × *V. riparia* cv. 3309 C) were measured. The resulting data were then used to simulate nitrate uptake efficiency and nutrient depletion as a function of root age.
• Both nitrate uptake and root respiration declined remarkably quickly with increasing root age. The decline in both N uptake and root respiration corresponded with a strong decline in root N concentration, suggesting translocation of nitrogen out of the roots.
• For simulations where no nutrient depletion occurs at the root surface, daily uptake efficiency was maximal at root birth and lifetime nitrate uptake efficiency slowly increased as the roots aged. Simulations of growth of roots into unoccupied soil using a solute transport model indicated the advantage of high uptake capacity in new roots under competitive conditions where nitrate availability is very transitory.

Key words: fine roots, grape, nitrate uptake, nutrient competition, root age, root efficiency, root respiration.

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Introduction

Shifts in root function with age have important implications for understanding nutrient uptake, root competition and optimal lifespan. Root systems function like a community of individuals in which some of the roots contribute more directly to water and nutrient uptake than others. Individual root elements often vary widely in their ability to absorb water and nutrients, depending on their order, age, and location in the soil. In this paper, we refer to roots devoid of lateral roots as 1st order, roots with a single set of dependent laterals as 2nd-order, and so on. First-order roots probably serve primarily for water and nutrient acquisition, while higher order roots may or may not contribute directly to the acquisition of water and nutrients. Many dicots have three or more orders of roots with mean diameters less than 2 mm (Pregitzer *et al.*, 1997, 2002).

Changes in root physiology with root age can strongly influence nutrient acquisition and competition. While it is widely recognized that new root growth into localized patches of nutrients may be advantageous in competition with other plants and microbes (Caldwell, 1994; Robinson *et al.*, 1999; Hodge *et al.*, 2000a; Hodge, 2004), there has been essentially no investigation of the physiological shifts of those roots as they age on a time-scale meaningful to understanding nutrient competition. Many soil nutrients are available for short

periods of time and enhanced uptake on the time scale of a day or less may strongly influence competitive outcomes (Jackson *et al.*, 1989; Hodge *et al.*, 2000b).

Changes in root physiology with age may also influence optimal root lifespan (Eissenstat & Yanai, 1997). Root efficiency of nutrient acquisition is influenced by not only nutrient acquisition, but also the resources expended in root deployment and maintenance. If plants preferentially retain the most efficient roots, then cost-benefit modeling can be used to explore how shifts in physiology affect optimal lifespan. Yanai *et al.* (1995) used a cost-benefit model to explore optimal root lifespan, with optimal lifespan being theorized as that point in a root's existence where lifetime efficiency was maximized in terms of the ratio of the amount of nutrients (or water) gained vs the amount of C expended. To date only limited data exist concerning nutrient uptake in relation to carbon costs over the lifespan of a root. Bouma *et al.* (2001) measured the effect of root age on respiration and P uptake in fine roots of apple and orange and found both declined rapidly with age. Modeling revealed that under the assumption of nonlimiting P supply, apple and citrus roots would become infinitely efficient as they age because declines in root carbon costs matched or exceeded the decline in nutrient (P) uptake.

The decline in P absorptive capacity with root age observed by Bouma *et al.* (2001) is consistent with observations on other species and for other resources. A number of these studies have demonstrated a decline in hydraulic conductivity or nutrient uptake with root age. Kramer & Bullock (1966) and Nobel *et al.* (1990) showed that older (pigmented) roots compared with young white roots often have diminished hydraulic conductivity. Pigmented roots also often exhibit diminished ability to take up calcium or potassium compared to young, white roots (Clarkson *et al.*, 1968; Atkinson & Wilson, 1980; van Rees & Comerford, 1990). Unlike Bouma *et al.* (2001), these studies did not provide a quantitative relationship between root age and the physiological process in question, because they generally did not compare roots of the same order and just differentiated between 'young' and 'old' roots. Thus, there is a very limited understanding of how the physiological activity of 1st-order lateral roots changes over time as they approach senescence. In particular, no studies to date have examined changes in physiology at a time resolution that includes the first couple of days of a root's life.

Another short-coming of almost all ecological studies of the physiology of root aging is the use of excised roots. While one may obtain reasonable estimates of the uptake of potassium using excised roots (Huang *et al.*, 1992), excision can be deleterious when estimating energy-intensive processes like nitrate uptake and assimilation (Bloom & Caldwell, 1988). Measurements of nitrate uptake by whole, intact root systems are not uncommon, but measurements of nitrate uptake by an intact lateral fine root of known age are unprecedented. Measurements involving such small root masses are difficult because they produce relatively small changes in external N concentration that are difficult to detect using a conventional approach in which depletion of the medium is monitored.

We used nutrient solutions of nitrate labeled with the stable isotope ^{15}N rather than nitrate depletion to estimate nitrate uptake rates as a function of root age for intact 1st-order grape roots. We also measured respiration rates of excised 1st-order grape roots of known age. Using the age dependent curves for nitrate uptake and respiration, we estimated daily and lifetime root efficiency to examine how the relative costs of nitrate uptake vary with root age. In addition, we measured root C and N concentrations as a function of root age to provide information on how age dependent changes influence fine root C and N economy. Lastly, we examined the implications of large rapid shifts in nitrate uptake capacity on potential competitive ability.

The 3309 C rootstock of grape (*Vitis rupestris* × *V. riparia* cv. 3309 C) is widely used in the wine and juice industry. It was chosen for its importance as a rootstock, previous work on respiration (Smart, 2004) and its relative large fine roots (which makes them easier to manipulate). More detailed information about root physiology may improve below-ground management of grape crops.

Materials and Methods

We conducted the experiments in two stages. First, the ^{15}N-labelled nitrate uptake method was verified as an acceptable surrogate for the method of allowing nitrate to deplete from the nutrient solution around grape roots (*Vitis rupestris* × *V. riparia* cv. 3309 C), as assessed via HPLC (Thayer & Huffaker, 1980, Fig. 1). Then a larger scale experiment was designed to measure both N uptake and respiration rates on grape roots of different ages.

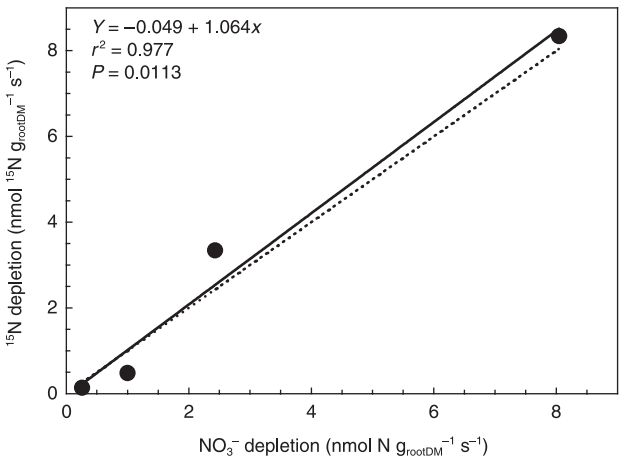

Fig. 1 Relationship between N-uptake as measured via NO_3^- depletion using HPLC and for N-uptake based upon net ^{15}N depletion. The measurements were performed on the same intact grape roots exposed to 0.1 mM nitrate as the sole N source. Solid line, regression line; dotted line, 1 : 1 ratio. The intercept with the *y*-axis was not statistically different from zero ($P = 0.929$).

Table 1 Characteristics of the fine grape roots used for the ^{15}N uptake and respiration measurements

		Age (d)	Dry mass (mg)	Length (mm)	Diameter (mm)
N uptake (n = 13)	Mean	13.3 (2.1)	0.32 (0.04)	25.1 (2.3)	0.51 (0.02)
	Min	0.5	0.08	8.1	0.41
	Max	23.0	0.60	41.9	0.75
Respiration (n = 19)	Mean	15.2 (3.3)	0.76 (0.09)	29.2 (0.9)	0.65 (0.03)
	Min	1.5	0.42	24.6	0.48
	Max	37.0	1.59	34.2	0.92

Numbers in parentheses are SE.

For the larger scale experiment, grape (*Vitis rupestris* × *V. riparia* cv. 3309 C) rootstocks were glasshouse-grown at The Pennsylvania State University in 7.6 l pots. Five rootstocks were planted individually in separate pots. A window of *c.* 20 cm by 15 cm (w × h) was cut in the pots and covered with transparent acetate film. The window was then covered with light-impenetrable shade cloth. New root growth was traced on the windows daily, with different color permanent markers. After 8 wk of root growth, an incision was made in the acetate, and substrate was gently washed away from a root of known age while keeping it attached to the plant. Two to four roots of each individual plant were used, 15 in total. Care was taken to avoid roots that either carried laterals or where age was uncertain. The exposed roots were allowed to hang into 0.4 ml Eppendorf vials filled with buffer solution of 10 mM MES, 1 mM $CaSO_4$ and 5 μM K_2HPO_4, pH = 5.7, and 0.1 mM unlabelled KNO_3. The tops of the vials were covered with Parafilm M and the window was covered with shade cloth. The roots were allowed to adjust to the buffered solution for at least 5 h. Each root was then gently washed with nitrogen-free buffer, and touched with an absorbent paper cloth to dry it and to remove organic specks of debris. Intact root tips 1- to 4-cm-long (Table 1) were inserted into a new vial filled with a known volume of buffer and 0.1 mM $K^{15}NO_3$ (99.6 atom% ^{15}N, Icon Services, Summit NJ, USA). The vial and the intact lateral root were then taped to the pot and covered with Parafilm M to prevent evaporation and desiccation. Vials containing solution devoid of roots were used as a control. The light impenetrable cloth was placed back over the window after the vials were in place.

Prior to all experiments, filter discs (Whatman #3) were washed with ultrapure water and then pretreated with 10 μl of unlabelled 0.25 M $(NH_4)_2SO_4$ and placed in tin capsules (9 × 5 mm, Environmental Microanalysis Ltd, Devon, UK). This ensured that each sample contained sufficient total N for accurate detection of the ^{15}N label following combustion and analysis using a continuous flow GC-IRMS (PDZ Europa, model Integra, Chesire UK, U.C. Davis Stable Isotope Facility). Aliquots (10 μl) were taken from the uptake solution at 0, 1, 3, and 5 h after root insertion and pipetted on separate pretreated filter discs. Roots were harvested after 5 h by cutting them at the edge of the original solution volume and rinsing them in distilled water. The volume change of the solution in each vial was determined by weighing the vial at the start of the measuring period and at the end and correcting for vial mass. On average, the starting volume was 0.45 ml, volume losses (other than those due to sampling) equaled 0.3 μl h^{-1} in the control vials and 11 ± 0.002 μl h^{-1} (mean ± SE) in the vials with a root. One root was removed from the analysis because it broke during the experiment. Roots were then scanned on a flatbed scanner with a transparency adapter and analyzed for color, length, and diameter (WinRhizo, Regent Instruments Inc., Quebec City, Canada). After scanning, they were dried at 65°C and their mass determined on a microbalance (Table 1). After correcting for background enrichment (unused filter discs with 5 μmol N at natural abundance) and volume changes (due to transpiration and sample removal), the decrease in ^{15}N content of the vial was calculated at each time point. Even though net nitrate uptake was not always changed by gentle handling, spinach roots were found to exhibit temporarily altered nitrate influx and efflux rates immediately after handling in a double labeling experiment (Ter Steege *et al.*, 1998). To reduce variation due to handling effects in our dataset, nitrogen uptake per unit root dry mass was calculated over the final 4 h. When nitrate uptake was calculated over the full length of the experiment, variation was considerably increased; nonetheless, the general trend of decreasing nitrate uptake with root age was still evident (data not shown).

The change in ^{15}N content in control vials was measured following the same exposure times as the experimental vials. Total uptake rate per root was corrected for changes observed in the control vials. The fluctuations in ^{15}N content of the control vials were a negligible fraction of total root uptake with the exception of some very old roots that had very slow uptake rates.

We initially tested this method on grape plants grown at UC Davis in growth chambers and found a good correlation between NO_3^- depletion measured using HPLC (Thayer & Huffaker, 1980) and nitrate uptake measured using this ^{15}N method (Fig. 1).

In addition to nitrate uptake, which was measured using this ^{15}N method, we also measured root respiration in a

temperature controlled (24.5°C) oxygen electrode system (Oxygraph, Hansatech, King's Lynn, UK). Intact 1st-order roots of known age were placed in vials with the same buffer used for N uptake measurements plus 0.1 mM KNO_3 and exposed for at least 30 min. Care was taken to avoid roots that either carried laterals or where age was uncertain. The roots were excised and rinsed, then immediately placed in a cuvette filled with stirred oxygenated buffer containing 0.1 mM KNO_3. Oxygen uptake was measured over at least a 15-min interval. After the respiration measurements were completed, the roots were rinsed, scanned, and dried. The respiration measurements were performed at a controlled temperature of 24.5°C. N uptake measurements were taken under ambient glasshouse conditions, where temperatures varied between 24 and 26°C.

Unused, traced roots of the grape plants were collected at final harvest and separated by age class. Multiple roots of the same age were pooled to obtain sufficient material (5 mg) for elemental analysis. Roots older than 25 d were rare, so two or more age classes were pooled and a mean age was assigned based upon the relative dry masses for each age class. The roots were dried to stable mass and analysed for C and N content on an elemental analyzer (UC Davis Stable Isotope Facility).

Multiple types of curves (linear, inverse 1st and 2nd order, exponential decay, hyperbolic decay, logarithm and rational decay), were used to describe N uptake or respiration as a function of root age. Each curve was fitted and tested for significance using graphics software (Sigmaplot 8.0) and statistical software (GenStat ver. 6.1), and the best (highest r^2 value), statistically significant ($P < 0.05$) fit was chosen.

Efficiency calculations

To calculate daily efficiency rates, equations derived from the experimental data for N uptake and respiration were extrapolated to 40 d, which is not an unusual lifespan for fine grape roots in the field as Anderson *et al.* (2003) has shown for a related grape species (*Vitus labruscana*). Daily efficiency of N uptake was calculated by dividing N uptake for day 0.5 by respiration rate for day 0.5, then for day 1 and for each day thereafter until day 40.

Lifetime efficiency was calculated by including the initial carbon concentration, 41.5 mmol C g^{-1} root dry mass, of the root at day 0.5 in the carbon costs. Respiratory carbon costs were estimated from O_2 consumption based upon an RQ of 1.1 (Bloom *et al.*, 1992). Cumulative nitrate uptake and cumulative carbon expenditure were calculated for each day, extrapolating the data to 40 d. Lifetime efficiency was defined as lifetime N acquired per lifetime C spent for any given root age.

Solute transport modeling

The influence of different rates of nitrate influx (I_{max}) was simulated using the Barber-Cushman solute transport model (ver. 3.6; Barber & Cushman, 1981; Barber, 1984). Nitrate influx at the root surface at a known soil solution concentration was estimated from the observed influx in the solution depletion experiments. Modelling was based on a newly emerged grape root 25 mm long, with a diameter of 0.51 mm and a mass of 0.31 mg growing in a soil solution with initial nitrate concentration in solution, C_{li} = 0.1 mM. Root growth rate was set equal to zero. Other parameters in the model were set at default values (effective diffusion coefficient, De = 2×10^{-6} cm^2 s^{-1}; buffer power, b = 1.0; water influx 10^{-7} cm^3 cm^{-2} s^{-1}, half distance between root axes 3.3 mm, K_m = 0.02 nM; and the minimum solute concentration for uptake to occur, C_{min} = 2 pM).

Results

Nitrate uptake, respiration and root N concentration

Nitrate uptake rates declined rapidly with root age, declining to 50% of the starting rate after a single day (Fig. 2a). The initial declines in respiration with root age were nearly identical to those of nitrate uptake (Fig. 2b). Respiration rates declined by 50% when comparing respiration rates of 0.5-d-old roots with those of 1.5-d-old roots and an additional 50% when comparing fitted respiration rates of 1.5-d old roots with respiration rates of 4-d-old roots. After day 4, measured respiration rates remained essentially stable at 40 nmol O_2 g^{-1} s^{-1}.

Considering the rapid initial decline in uptake and respiration with root age, we also analysed the decline in N uptake rate and respiration for roots older than 11 d. We found no significant relationship between either respiration or N uptake with increasing root age for roots older than 11 d. Mean N uptake for roots older than 11 d was 3.45 nmol ^{15}N g^{-1} s^{-1}, and mean root respiration was 43.8 nmol O_2 g^{-1} s^{-1}.

Root nitrogen concentrations also decreased rapidly with root age (Fig. 3), with 3-d-old roots having half the nitrogen concentration of 1-d-old roots.

Root efficiency and optimal root lifespan

Daily efficiency of nitrogen uptake was described by a declining hyperbolic function with a highest daily efficiency of 0.0779 mol N mol^{-1} O_2 respired for newly born roots (Fig. 2c). N uptake decreased marginally faster than respiration rate did for the first 40 d (Fig. 2a,b). At that point, daily efficiency had decreased to 0.0753 mol N mol^{-1} O_2 and further extrapolations to 1000 d show that daily efficiency had essentially stabilized at that value. Most of the small drop in efficiency rate was achieved in the first 10 d of root life (Fig. 2c). Cumulative daily N uptake and carbon expenditure allowed us to calculate a lifetime efficiency of nitrogen uptake at any given root age under conditions of no nitrate depletion at the root surface (Fig. 4). In plotting the resulting lifetime

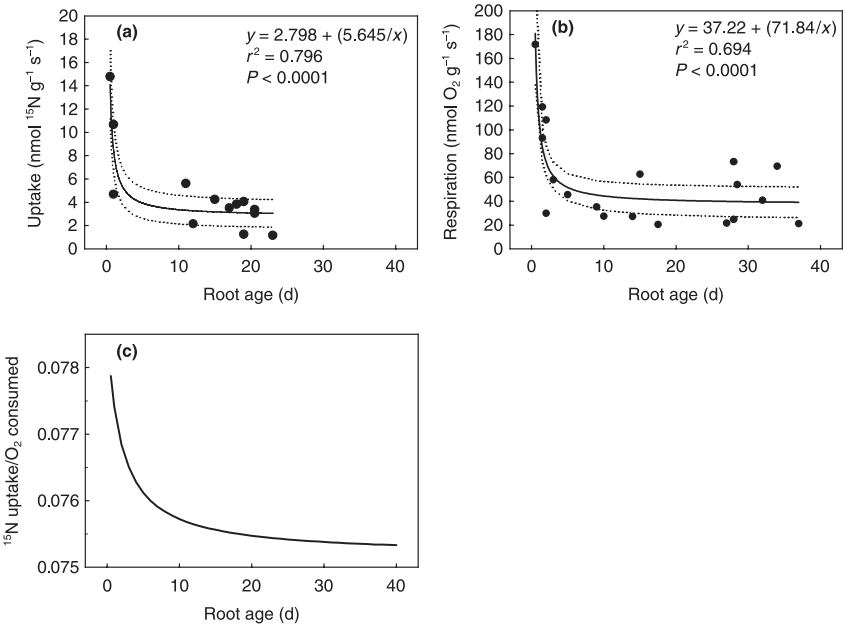

Fig. 2 Nitrogen uptake, respiration and efficiency of intact 3309 C grape rootstock fine roots as a function of root age. (a) N uptake (nmol ^{15}N g_{rootDM}^{-1} s^{-1}) intercept SE is 0.59 and the SE for a is 0.86 (b) respiration (nmol O_2 g_{rootDM}^{-1} S^{-1}), intercept SE is 6.27 and the SE for a is 11.56 and (c) daily nitrate uptake efficiency (nmol ^{15}N nmol O_2^{-1}). Dotted lines represent 5% and 95% confidence intervals for the regression line.

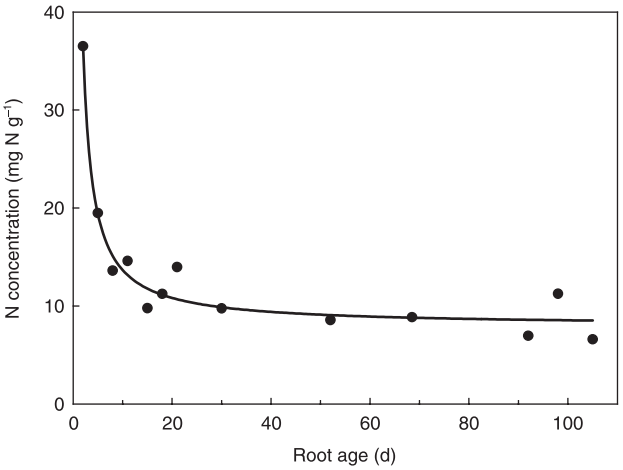

Fig. 3 Nitrogen concentration of 3309 C rootstock roots of different ages. Roots were traced on acetate windows to assess root age (see the Materials and Methods section). When necessary, roots of close age classes were pooled and a mean age was assigned based upon root mass of each age class in the sample. Pooling was not necessary for roots < 25 d old.

nitrogen uptake efficiency rate, we found that lifetime N uptake efficiency initially keeps increasing with root age (Fig. 4b). After 40 d, a lifetime efficiency of 0.0565 mol N mol^{-1} C is reached. Again, lifetime efficiency had essentially stabilized by then.

Simulated nitrate uptake of a new root in soil

Simulation modeling (Barber & Cushman, 1981) of the different uptake rates indicated that an initial high uptake by roots when they first enter new soil volumes may be advantageous in rapidly acquiring a transitory resource (Fig. 5). Differences in simulated nitrate influx among roots with different maximum potentials for nutrient uptake (I_{max}) were clearly evident for about 10^3 s (17 min) but diminished by 10^4 s (2.8 h). After little more than 1 d, most of the nitrate had been depleted for all three values of I_{max} under the simulated conditions where competing roots were spaced 6.6 mm apart. Nitrate depletion zones that form around a root were also simulated under different values of I_{max} (Fig. 5b). One hour after a simulated grape root had grown into new soil, the initial nitrate concentration was depleted 25, 39 and 44% at 0.5 mm distance from the root for roots corresponding to uptake potentials of 5, 10 and 15 nmol g^{-1} s^{-1}, respectively.

Discussion

Nitrate uptake and respiration

Both nitrate uptake rate and respiration rate were found to decrease rapidly as fine lateral roots of grape aged (Fig. 2). Nitrogen uptake rates and respiration rates declined to half within only 2 d of emergence. This rapid decline is an interesting and surprising result, given a median lifespan of grape roots of a related species of 50 d or more (Anderson *et al.*, 2003). The most important implication of this result would be in competition for very ephemeral resources (Fig. 5). Simulation modeling suggested that nitrate acquisition would be substantially enhanced for the first couple of hours after a new root entered undepleted soil if it had high uptake capacity. This rapid depletion would allow for effective competition with plants and perhaps microbes. Robinson *et al.* (1999) and Hodge *et al.* (1999), for example, showed

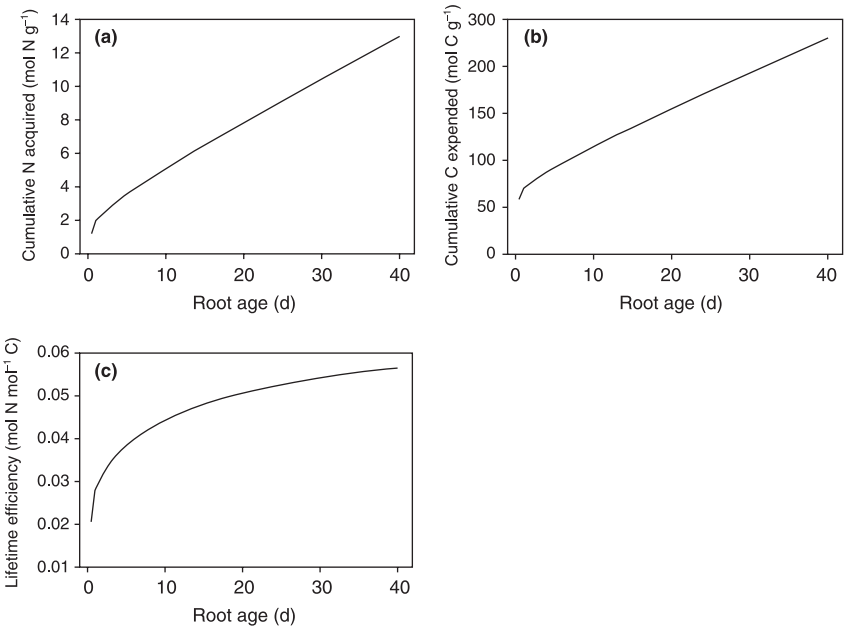

Fig. 4 Age-related lifetime nitrogen uptake efficiency of 3309 C grape rootstock fine roots. (a) Cumulative nitrogen uptake, (b) cumulative carbon expenditure and (c) lifetime N uptake efficiency of a grapevine root. Carbon cost was calculated from oxygen consumption assuming an RQ of 1.1 and root carbon concentration was included in the carbon costs at age 0.5 d as 41.50 mmol C g^{-1} root dry mass.

that rapid uptake from root proliferation in patches could confer an important competitive advantage. While they identified the importance of a new absorptive surface as an important contributor to the effectiveness of the more competitively successful grass, enhanced uptake kinetics associated with the new roots may have also played a role. Thus, rapid uptake potentials could be advantageous at rapidly depleting the nitrate before other roots or microbes were able to acquire the nutrients.

From an evolutionary perspective it would be unfavorable to maintain a high N concentration and a high nitrogen uptake capacity with associated high respiration rates when all available nitrogen has been acquired. Likely, grape root systems respond to the appearance of high nutrient patches via a burst of new root growth as commonly observed in heterogeneous soil environments (Robinson, 1994). These young roots will have very high capacities for N uptake so that they can deplete the patch before competing organisms do so.

The very young roots also exhibited very high respiration rates, however, we have no mechanism to establish whether these high respiration rates were truly associated with high nitrogen uptake rates or due to high growth rates in the very young roots. For older roots, we know the tips had not elongated since they were traced last, thus those roots had not shown signs of any further growth. Accordingly, for roots older than 1 d, we can reasonably presume that most respiration was for nutrient uptake and maintenance and not for growth. Newly born roots, on the other hand, exhibit high nitrate uptake rates, high respiration rates, and a high N concentration (Figs 2 and 3). The very youngest roots may store the acquired nitrate without assimilating it further and such nitrate may serve as an osmoticant that drives root cell expansion in the elongation zone near the root apex (Bloom *et al.*, 2002). A high nitrogen concentration in the young roots (Fig. 3) is consistent with this interpretation. This pool of N may subsequently be diluted with growth or translocated to other roots or to the shoot. The continued decline of root N with age in roots older than 1 d (Fig. 3) argues for translocation of N out of these roots as well. When we studied the two sets of roots used for respiration and nitrate uptake further (as the roots used for C and N analysis had already been combusted), we found no correlation between root age and specific root length (SRL) or diameter in those two data sets. Nor did we find a relationship between root age and C concentration. Therefore, these findings support that the decline in N concentration was due to nitrogen moving out of the roots, rather than a dilution effect.

Efficiency of nitrate uptake

Daily efficiency, the ratio of daily nitrate uptake over daily respiration, provides an index of how the physiology of a root changes in terms of N uptake and respiration over time. Compared with lifetime efficiency, daily efficiency does not take into account the cost of root construction, and therefore is not a good term in modeling how root deployment may be optimized for nitrate acquisition. However, daily efficiency does give a good instantaneous measure of the efficiency of the actual nitrogen uptake process. The highest daily efficiency for newly born roots was 0.078 mol N absorbed mol^{-1} O_2 respired, and declined thereafter to a low of 0.075 mol N mol^{-1} O_2 after 40 d, a change of only 3.8%. It was expected that daily uptake efficiency would decline with root age, however, estimated daily uptake efficiency was essentially stable.

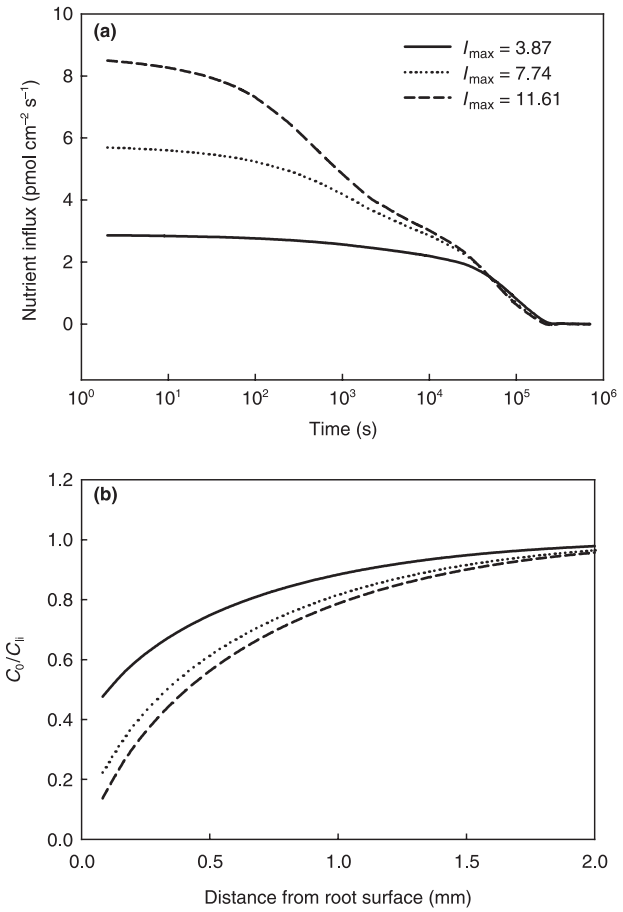

Fig. 5 Model simulations of the effect of different maximum rates of nitrate uptake on total nitrate uptake and rate of soil depletion using the Barber-Cushman solute transport model (ver. 3.6; Barber & Cushman, 1981; Barber, 1984). (a) Nitrate influx as a function of time since root entered soil volume for three different maximum NO_3^- uptake rates (5, 10 and 15 nmol g^{-1} s^{-1} corresponding to 3.87, 7.74 and 11.61 pmol cm^{-2} s^{-1}). Modelling based on a newly emerged grape root 25 mm long, with a diameter of 0.51 mm and a mass of 0.31 mg growing in a soil solution with initial nitrate concentration in solution, C_{li} = 0.1 mM. Root growth rate was set equal to zero. Other parameters in the model were set at default values (effective diffusion coefficient, De = 2×10^{-6} cm^2 s^{-1}; buffer power, b = 1.0; water influx 10^{-7} cm^3 cm^{-2} s^{-1}, half distance between root axes 3.3 mm, K_m = 0.02 nM; and the minimum solute concentration for uptake to occur, C_{min} = 2 pM). (b) Depletion zone development 1 h after a grape root has entered a new soil volume for three maximum nitrate uptake rates. Nutrient concentration of the solution various distances from the root surface are expressed as the ratio of solute concentration at that soil location (C_0) divided by initial solute concentration, C_{li} (0.1 mM nitrate).

Rates published earlier for ion uptake costs in grasses ranged from 0.43 to 4 mol anions mol^{-1} O_2 (Poorter *et al.*, 1991). If we assume a similar maintenance respiration for grape roots as for the *Carex* species used by Van der Werf *et al.* (1988), 6.4 nmol O_2 g^{-1} s^{-1}, daily uptake efficiency for nitrate in our grape roots is 0.08 mol nitrate mol^{-1} O_2. Poorter *et al.* (1991) then assumed a fixed uptake rate of NO_3^{-1}: to other anion uptake of 3 : 1, which in our case would lead to 0.10 mol anions acquired mol^{-1} O_2 respired. There are four important differences between our study and the Poorter *et al.* (1991) study that might explain the 4–40 fold difference between their values and our values for anion uptake respiration. First, we used a woody plant species that may have slower growth rates or higher maintenance respiration rates. Second, in our experiment we used 0.1 mM N, rather than 2 mM N in the solution, thus changing the energetics of uptake. Third, our plants were grown in soil, rather than in hydroponic solution and thus may further differ morphologically. Finally, we measured depletion of ^{15}N labeled nitrate from the solution, rather then net nitrogen uptake rate based upon changes in tissue nitrogen concentrations and mass increases.

As a woody plant species, grape could have higher maintenance respiration rates than sedges, which is not accounted for when we subtract the maintenance respiration as measured in the *Carex* species. The much lower NO_3^- concentration used in our experiment could have caused our plants to use a much more energy intensive transport system than the hydroponically grown grasses supplied with a high N concentration. For instance, at 100 μM, the grape roots would have been using the constitutive high affinity NO_3^- transport system (cHATS, Crawford & Glass, 1998), whereas at 2000 μM the hydroponically grown grasses most likely were within the range of the low affinity transport system (LATS). Possibly our 5-h pretreatment time was not enough to induce the inducible high affinity pathway (IHATS) in our plants. For example, in *Picea glauca*, it was necessary to expose roots to NO_3^- for 3 d in order to achieve peak $^{13}NO_3^-$ influx (Kronzucker *et al.*, 1995). This may explain why our anion uptake efficiency rates are so much lower than those reported by Poorter *et al.* (1991). As we measured nitrate influx in our experiment, rather than net nitrate uptake, actual costs of net nitrate uptake may even have been higher than estimated.

Unfortunately, we were unable to acquire any data on N-uptake of roots aged 2–10 d. The lack of these data means that we cannot be certain of the shape of our N uptake curve; however, we note the inverse first-order curve provided the best, statistically significant, fit to the data we have and small changes in the parameters of the equation did influence our efficiency calculations. We found that, with slightly altered parameters, daily efficiency either increased or decreased with age, depending on the choice of equation fitted. These equations had either a less good fit, or nonsignificant parameters. Changes over time were generally minor, and the major point that daily efficiency was essentially unaffected by root age, remains valid. When actually dividing the N uptake of a 0.5-d-old root by the respiration rate of a 0.5-d-old root, we find an efficiency of 0.086 N per unit O_2 respired, instead of 0.078 as predicted by dividing the fitted equations. Similarly we underestimated the daily efficiency of roots older than 11 d which was 0.079 N per unit O_2, rather than the 0.075 N per

unit O_2 predicted by our models. The error in these two numbers, however, is relatively small, 9.7% and 4.4%, respectively.

Lifetime efficiency

Simulations for P absorption suggested that supply rate was the most important factor determining lifetime P uptake efficiency so that roots exposed to an unlimited P supply would have infinite lifetime efficiency (Bouma *et al.*, 2001). Our data on lifetime N uptake efficiency follow a similar pattern. In terms of N uptake efficiency there would be no advantage in shedding an old root, provided that N remains in ample supply. Were these roots located in a natural soil environment, nitrate supply normally would diminish due to the formation of nutrient depletion zones around the root (Fig. 5b). However, in agricultural soils the availability of nitrate in the soil solution is generally high (Wolt, 1994, but see Crawford & Glass, 1998). It is possible a nutrient other than nitrate (e.g. phosphate or ammonium) is more important in determining root lifespan, potentially explaining the lack of an optimum root lifespan in our experiment. In the field, fine roots of mature grape vines have been observed to typically exhibit median lifespans of *c.* 50–100 d (Anderson *et al.*, 2003). Field observations show that root diameter, soil depth and the timing of root emergence all have strong influences on grape root lifespan (Anderson *et al.*, 2003). Similar observations have been made in peach and apple (Wells & Eissentat, 2001; Wells *et al.*, 2002). The direction and size of the effects on lifespan can vary by season and/or year. Root longevity may also be controlled to some extent by other external factors such as soil microorganisms. For example, median lifespan of sugar maple roots was extended by more than 59 wk by the addition of fungicide and by the addition of fungicide combined with insecticide (Eissenstat *et al.*, 2000). Soil microorganisms not only play a role in root herbivory, but also influence nutrient cycling through the soil and the potential availability of these nutrients to the plant. Clearly, a major challenge for understanding root ageing processes in plants will involve teasing apart the various environmental and physiological factors that contribute to the ageing responses.

In a natural environment, where a plant must compete with its neighboring plants and soil organisms, fitness is not solely determined by the efficiency of resource capture, but also by the effectiveness (i.e. the amount) of resource capture. A high nitrate uptake capacity would ensure uptake of nitrate before other organisms acquire it, thus possibly explaining the very high uptake capacity when roots are born. Our data show that respiratory costs for older roots are low. Roots older than 9 d in our study only exhibited 25% or less of the respiration rates measured in newly born roots. With the old root already there, new roots can then be grown quickly to take advantage of new quantities of nutrients as they become available later. New roots growing from apparently 'dead' fine roots are a common occurrence within woody fine root systems. However, in times of high nutrient demand or when competing for nutrients that are scarce, it could be a more viable strategy to abandon roots with diminished uptake rates in favor of newly emerged roots that are capable of high uptake rates. Plants could be employing both strategies, explaining why some species exhibit short median root lifespans, yet also appear to have a sizeable fraction of fine roots that do not die within the sometimes lengthy measuring periods employed by several researchers (Burton *et al.*, 2000; Wells *et al.*, 2001; Anderson *et al.*, 2003; Matamala *et al.*, 2003).

In conclusion, our results suggest that the initial high uptake capacity of roots may be an important contributor to competitive effectiveness. As fine roots age, the advantages of high uptake capacity diminish and, the associated costs of maintaining a high affinity and capacity for nitrate uptake were reduced. Since both N uptake and cost reductions occur simultaneously, there is little effect on root efficiency. The primary cause for a decrease in lifetime efficiency of nitrate uptake would be depletion zones developing around the root, thus implying that soil properties and soil moisture may have a greater effect on lifetime efficiency and root lifespan than decreases in root uptake kinetics with age. Clearly, these results warrant further investigations that include measurements of other nutrients (e.g. phosphate and ammonium).

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