Estimating age-dependent costs and benefits of roots with contrasting life span: comparing apples and oranges

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Summary

- The relation between root age and root function is poorly understood, despite its importance to root longevity.
- The effect of root age on respiration rates and ^{32}P-uptake kinetics was determined for roots excavated from mature apple and citrus trees (median root life spans of 30 vs 300 d). To evaluate whether root longevity maximizes the efficiency of nutrient capture, daily and lifetime efficiencies were calculated by dividing simulated P-uptake benefits (solute transport model) by age-specific respiration costs.
- We found that: respiration rates and P uptake capacity change with root age in a species-specific way; and soil characteristics that determine the rate of nutrient depletion in the rhizosphere are as important as changes in root physiology in determining the age at which a root reaches its maximum efficiency.
- Further testing of the efficiency of nutrient capture as a predictor of root life span requires measurement of both soil properties and age-specific physiology of roots including their mycorrhizal fungi.

Key words: apple, citrus, minirhizotron, ^{32}P, respiration, phosphorus uptake, root life span, root turnover, root age, simulation modelling, uptake kinetics.

Introduction

Root longevity has important consequences for plant growth and productivity, plant competition, and carbon and nutrient cycling at the ecosystem scale. In various ecosystems, net primary production is greater below ground than above ground (Caldwell, 1987; Santantonio & Grace, 1987; Gower *et al.*, 1994). Root turnover may return four to five times more carbon to the soil than above-ground litter (Lehmann & Zech, 1998). Consequently, there is a need to include the effects of root turnover in models of carbon and nutrient cycling (Cox *et al.*, 1978; Vogt *et al.*, 1986; Hendricks *et al.*, 1993; Jackson *et al.*, 1997; Norby & Jackson, 2000). However, quantitative information on root lifespan is scarce, as the techniques involved (including minirhizotron observations, sequential coring) are extremely time consuming and turnover rates vary with species and environmental conditions (Pregitzer *et al.*, 1993; Fahey & Hughes, 1994; Watson *et al.*, 2000). Compared with our knowledge on the turnover of above-ground tissues (Reich *et al.*, 1997), little is known about the mechanisms controlling root turnover, and ideas for a quantitative theory have only recently started to emerge (Eissenstat & Yanai, 1997). We suspect that the key to a better understanding of the mechanisms controlling root turnover is likely to be found in the still poorly-defined relation between root function and root age.

Yanai *et al.* (1995) introduced a cost-benefit model to explore whether root life span maximizes the efficiency of nutrient capture, with efficiency defined as the ratio of nutrient gained (benefit) per unit carbon expended (cost) on a root-mass basis. The optimal life span is that which maximizes this ratio. The cost-benefit model uses the concept that a root system consists of a population of individuals, each with its own life history (Harper, 1977). Individual roots of different ages are likely to have different physiological characteristics and, as

a consequence, have different nutrient benefits and carbon costs. Controlling the age structure of the root system by turnover may be a mechanism by which plants control their efficiency at exploiting the soil (Caldwell, 1979). Testing the cost-benefit model has, however, been limited by the lack of age-dependent plant parameters for nutrient uptake and carbon expenditure. Uptake kinetics have been related to various factors other than age, such as a root being woody or not (Van Rees & Comerford, 1990). The age-related data that are available on uptake kinetics are often indirect, as they are based on measuring capacities at various distances from the root tip. In addition, these studies are of limited relevance for testing the cost-benefit model, as they have been done mainly on young, often seminal roots of agricultural species (Clarkson, 1991). The age-dependency of root-respiration rates has not yet been established for woody tree species. Observations are available for three desert succulents (Palta & Nobel, 1989a,b) and for grape (Comas *et al.*, 2000).

Our first objective was to study the age dependence of ion uptake capacity and respiration of fine roots. We used mature trees of two species with contrasting root characteristics: apple ('Red chief Delicious' on M26 rootstock) and citrus (Red grapefruit on sour orange rootstock). Age-dependent values of phosphorus uptake kinetics, respiration rates, and carbon content were determined on excised roots. We applied these rates to determine the age at which roots maximize the efficiency of nutrient capture, defined as the ratio of P uptake to C cost. Finally, observed root life spans for the two species were compared with that predicted by the theoretical model to test the cost-benefit model that predicts root life span based on the efficiency of nutrient capture (Eissenstat & Yanai, 1997).

Materials and Methods

Plant material

We studied roots of mature apple trees (*Malus domestica* Borkh.) (Red chief Delicious on M26 rootstock; 20-yr-old) in an orchard at the horticultural farm of the Russell E. Larson Agricultural Research Center, Pennsylvania State University, PA, USA (40°85′ N, 77°83′ W). Trees were about 2.5 m tall and planted at a 2-m spacing in a 'Penn State four-wire low-hedgerow' trellis system with 3.7-m spacing between rows. Soil at this location is Hagerstown silt loam, a Typic Hapludalf. Apple trees had not been fertilized with either N or P for several years before the study because trees did not show any signs of nutrient deficiency, and production was not apparently nutrient limited. In Florida, USA, we studied roots of 9-yr-old bearing red grapefruit (*Citrus paradisi* Macf.) trees on sour orange rootstock (*C. aurantium* L.) at the University of Florida, Citrus Research and Education Center (27°22′ N, 80°32′ W). Trees were about 3.4 m tall with a 3.3-m canopy diameter, planted at 2.4-m spacing within the row with 5.4 m between rows. Soil at this location is Candler fine sand, a Typic Quartzipsamment. Trees were fertilized four times a year with about 400 kg N and 200 kg P per ha. This was sufficient to prevent nutrient limitation to production. In apple (1996) and citrus (1995 and 1996), root longevity was determined on six trees, each surrounded by eight clear butyrate minirhizotron tubes. Turnover rates were obtained by analysing sequential video images from minirhizotrons (Eissenstat *et al.*, 2000).

Excavating roots of different ages

The age dependency of respiration, phosphorus uptake capacity, and elemental composition of roots was determined on root segments followed since birth using root boxes buried in the soil ($0.6 \times 0.5 \times 0.4$ m deep) containing an acetate window (0.55×0.30 m) on the side of the box facing the tree. The boxes were installed about 0.5 m from the bole of the trees, and the air gap in front of the window was filled with sieved soil to obtain good contact. To minimize temperature fluctuations and shield the roots at the acetate window from light, a plate of 50 mm-thick Styrofoam was put against the window and the root boxes were covered when not in use. For apple (1996), root boxes were installed on 20 trees, whereas for citrus (1997) 10 root boxes were placed at the same six trees previously used for the minirhizotron study.

At 1- to 2-wk intervals, new roots were traced on the acetate window, using coloured markers to track the age of individuals. Apple roots were traced beginning 25 June 1996, 8 wk after installing the boxes, to allow the roots time to establish. The citrus roots were given 3 wk to establish before the first tracing on 25 February 1997. After identifying a population of roots of known ages, individual roots were excised for physiological measurements and chemical analysis by cutting through the acetate window. During transport to the lab, excised roots were kept in 1-mM $CaSO_4$ solution buffered with 5-mM MES adjusted to pH 5.5 with 1 M KOH. In the lab, roots were washed free of soil, using the same $CaSO_4$: MES buffer.

Age-dependent respiration rates and carbon content

Root respiration rates were measured in a 3-ml cuvette (10 mm ID) at 25°C, using a Clark-type oxygen electrode (standard Hansatech electrode disc) connected to a CB1D control box (Hansatech Instruments Ltd, Norfolk, UK). Respiration was measured on apple roots aged 1 ($n = 3$), 7 ($n = 3$), 14 ($n = 6$), 21 ($n = 3$), 28 ($n = 5$), 32 ($n = 4$), 35 ($n = 5$), and 38 ($n = 6$) d. For citrus, we used roots aged 4.2 ($n = 4$), 11.8 ($n = 6$), 22.7 ($n = 6$), 35.6 ($n = 7$), 51.8 ($n = 9$), 71 ($n = 3$), and 80 ($n = 1$) d. After the measurements, roots were oven dried (70°C) and weighed. Chemical analysis was limited by the small biomass of root material that could be harvested from the root boxes. In apple, roots of known age were freeze-dried at –60°C for 72 h and analysed for C and N content (Fisons Elemental Analyser EA1108, CE Elantech, Inc., Lakewood, NJ, USA). For citrus, only samples of unknown age were analysed for C and N content.

Age-dependent P uptake

Phosphorus uptake by excised apple and citrus roots was determined using the tea-bag technique (Epstein *et al.*, 1963). Roots of known ages were placed in 2.9-cm diameter plastic cassettes with 1.6-mm holes (Histo-prep tissue capsules, Fisher Scientific, Pittsburgh, PA, USA). Cassettes were immersed in $CaSO_4$: MES solutions with different concentrations of P (1, 5, 20, 50, 100, and 1000 μM), which reflect the range of concentrations that might occur under natural and agriculturally ameliorated field conditions, including fertilizer bands in orchards. We commonly used two replicates per root age (in total 7 age classes for citrus and 15 for apple) per P concentration (in total six P concentrations), with a range of one to four. The total number of samples was thus around 80 for citrus and 180 for apple. In each experiment, all bottles contained an equal amount of ^{32}P label dissolved in an equal volume. The amount of label (195–270 μCi) and the uptake period (10–14 min) varied slightly between experiments. In all experiments, the volume in the bottles (250–300 ml) was sufficient to guarantee that all cassettes were submerged. To remove ^{32}P adhering to roots, we rinsed twice (3 min each) with non-labelled solution. Phosphorus concentrations used for rinsing were kept similar to those used during uptake, as theoretically, this would result in the same relative error (i.e. P still adhering after rinsing) for each P concentration. During both labelling and subsequent rinsing, the medium in the bottles was vigorously mixed by aeration. After rinsing, roots were removed from the cassettes and oven dried, weighed, ashed and dissolved in 10 ml 100 mM HCl in which we counted ^{32}P beta emissions (5–1700 KeV; Packard Tri-Carb 1500 Liquid Scintillation Analyser, Packard Instrument Company, IL, USA).

We derived relative uptake rates for each P concentration by (*i*) identifying per P concentration the age group with the highest maximum uptake rate and (*ii*) expressing the uptake rate of all other age groups as a fraction of that maximum uptake rate. The relative uptake rates for all P concentrations were then combined and plotted against root age. We subsequently pooled age groups with similar relative uptake rates, for determining the uptake kinetics within those age groups. The uptake kinetics of apple and citrus were described by Michaelis-Menten parameters, as the data were insufficient to fit a multicarrier system model (Nissen, 1991). We simulated the variation in uptake rates with root age by varying the value of I_{max}. Previous sensitivity analyses have shown that simulated nutrient uptake is especially sensitive to this parameter (Williams & Yanai, 1996).

Root density and water uptake rate by the roots

Root length density, the length of root per unit soil volume (L_V), was determined by taking soil cores (5 cm diameter). For apple, cores were taken in August and September of 1997 (n = 12); for citrus, cores were taken during November of 1996 (n = 16). To determine overall root length and the average root radius (r_0), roots were washed from the soil and stained for 1 h with 0.16 g l^{-1} neutral red dye (Sigma Chemical Co., St. Louis, MO, USA). The stained roots were scanned using a flat bed scanner (HP ScanJet II, Hewlett Packard, USA). Root length and the average root radius were calculated using image analysis software (Delta-T SCAN, Delta-T Devices Ltd, Cambridge, UK). Roots and the soil from each soil core were dried at 70°C and weighed, to calculate bulk density and specific root length.

Water uptake rates were estimated by placing rain-out shelters (1.2 by 1.8 m) over parts of the soil volume where the trees were rooted, while monitoring changes in soil moisture content. In apple, rain-out shelters were placed from 25 July 1997 till 17 September 1997, whereas in citrus rain-out shelters were placed from 5 July 1996 to 16 September 1996. The drought treatment we induced by the rain-out shelters gave a reasonable estimate of water uptake by the tree, as the decrease in soil water content was approximately linear (data not shown). Soil moisture was monitored continuously using time domain reflectometry probes (TDR; 1502C metallic time domain reflectometer; Tektronix Inc., Beaverton, OR, USA) connected to a datalogger CR 21x (Campbell Scientific Inc., Logan, UT, USA) (Topp & Davis, 1985; Topp, 1993). Probes were inserted at different depths in the soil. Signal analysis used software developed by R. Hubbard (Department of Soil Physics, Utah State University, UT, USA). Combining soil moisture with root density data, we calculated the water uptake rate by the roots (v_0; $cm^3\ cm^{-2}\ s^{-1}$):

$$v_0 = \Delta\,\theta_{Vol.}/[100 \times \Delta\ \text{time} \times L_V \times 2\,\pi\,r_0] \qquad \text{Eqn 1}$$

(L_V, the root length density ($cm\ cm^{-3}$); $\Delta\,\theta_{Vol.}$, the change in volumetric soil water content (%) over a given dry down period (Δ time; d); and r_0, the root radius (cm).) The volumetric soil water content (θ_{vol}; $cm^3\ cm^{-3}$) and the uptake rate of water (v_0; $cm^3\ cm^{-2}\ s^{-1}$) (Table 1) are parameters required for simulating nutrient uptake by the steady-state solute transport model (Yanai, 1994).

Parameterizing the model for calculating the efficiency of nutrient capture

In the cost-benefit model that explores whether root life span maximizes the efficiency of nutrient capture (Yanai *et al.*, 1995; Eissenstat & Yanai, 1997), efficiency is defined as the ratio of nutrient gain to carbon expenditure. Efficiency may be calculated either on a daily basis (E_{daily}; mmol P [mol C]$^{-1}$) or over the life of a root ($E_{lifetime}$; mmol P [mol C]$^{-1}$):

$$E_{daily} = UPTAKE_{daily}/COST_{daily} \qquad \text{Eqn 2}$$

$$E_{lifetime} = UPTAKE_{cumlative}/COST_{cumlative} \qquad \text{Eqn 3}$$

Table 1 Parameters used in calculating the efficiency of nutrient capture. The buffer capacity (b) is calculated as $\theta + \rho K_d$ and the effective diffusion coefficient (D_e) is $D_l \theta f_l/b$. The impedance (f_l) is $3.13 \times \theta^{1.92}$ (Van Rees *et al.*, 1990)

			Apple, Hagerstown silt loam		Citrus, Candler fine sand	
Parameter	Symbol	Units	Estimate	Source of data	Estimate	Source of data
Plant parameters affecting Carbon expenditure						
root carbon content	C_{root}	mmol C g^{-1}	38.5 ± 0.19 (*n* = 39)	excavated roots 1996	35.3 ± 0.57 (*n* = 86)	excavated roots 1997
median lifespan	L	days	30	minirhizotrons (Fig. 1)	300	minirhizotrons (Fig. 1)
root respiration rates	$R_{observed}$	nmol C g^{-1} s^{-1}	Fig. 4	excavated roots 1996	Fig. 4	excavated roots 1997
Plant parameters affecting Phosphorus uptake						
specific root length*	λ	cm g^{-1}	9300 ± 1200	Eissenstat *et al*. (2000)	1824 ± 144	Eissenstat *et al*. (2000)
root radius*	r_0	cm	0.014 (*n* = 5)	soil cores 1997	0.0335	Eissenstat (1991)
half length to next root*	r_x	cm	2.536	soil cores 1997	0.471	soil cores 1996
root length density	L_V	cm cm^{-3}	0.049 ± 0.017 (*n* = 12)	soil cores 1997	1.43 ± 0.10 (*n* = 16)	soil cores 1996
uptake rate of water* (< = > radial water velocity)	v_0	cm^3 cm^{-2} s^{-1}	4.45×10^{-6}	Eqn 1; dry down 1997	2.23×10^{-8} 2.63×10^{-8}	Eqn 1; dry down 1996 Eissenstat (1991)
half-saturation constant*	k_m	µmol cm^{-3}	0.077	excavated roots (Fig. 3)	0.102	excavated roots (Fig. 3)
maximal uptake rate*	I_{max}	µmol cm^{-2} s^{-1}	1.22×10^{-6} to 8.64×10^{-8}	excavated roots 1996 after Figs 2 and 3	1.46×10^{-6} to 5.05×10^{-7}	excavated roots 1997 after Figs 2 and 3
Soil Parameters affecting Phosphorus uptake						
buffer capacity*	b	–	176.2	# #	4.52	Eissenstat & Yanai (1997)#
P in soil solution*	C_{av}	µmol cm^{-3}	0.65	estimated at 20 p.p.m.	0.54	soil samples CREC
effective diffusion coef.*	D_e	cm^2 s^{-1}	4.96×10^{-5}	# #	4.10×10^{-4}	# #
diffusion coef. in H_2O**	D_l	cm^2 s^{-1}	8.79×10^{-2}	CRC HANDBOOK	8.79×10^{-2}	CRC HANDBOOK
impedance factor**	f_l	–	0.324	# #	0.026	# #
slope adsorption isotherm**	K_d	cm^3 g^{-1}	120.5	Wolf (1988)#	3	Eissenstat & Yanai (1997)#
soil bulk density	ρ	g cm^{-3}	1.46 ± 0.02 (*n* = 83)	@	1.48	Eissenstat & Yanai (1997)#
Vol. soil water content*	$\theta_{vol.}$	cm^3 cm^{-3}	0.307 ± 0.007	54-d period 1996	0.082 ± 0.001	60-d period 1996

*Parameters used in simulating nutrient uptake. **Data used to calculate those parameters. # Literature citations are soil specific. # # Data are calculated according to the equations that are given in the heading of the table. @ Penn State University Soil Characterization Database System, available at *http://www.personal.psu.edu/f8i/pedon*.

($UPTAKE_{daily}$ and $COST_{daily}$, the daily rates of nutrient gain (μmol P g^{-1} d^{-1}) and carbon expenditure (mmol C g^{-1} d^{-1}); and $UPTAKE_{cumlative}$ and $COST_{cumlative}$, the nutrient gain (μmol P g^{-1}) and carbon cost (mmol C g^{-1}) accumulated since the birth of the root.) The $COST_{cumlative}$, at d 0 is estimated as the root C content. In all cases, integration over time was done with a time step of 1 d. The root lifespan that maximizes efficiency can be visualized by plotting $E_{lifetime}$ over time, and represents the theoretically optimal root life span (Yanai *et al.*, 1995; Eissenstat & Yanai, 1997).

Parameters for calculating nutrient uptake

Phosphorus uptake (UPTAKE) was estimated by three alternative methods. In the first (M1), roots are supplied with unlimited P, so that the P-uptake rate equals the age-dependent value of I_{max}. By method two (M2), P-uptake rate equals the age-dependent value of I_{max} multiplied by a fraction that decreases proportionally from 1 to 0 over a fixed period. Finally (M3), the P-uptake rate is estimated using a steady-state model of solute uptake (Nye & Tinker, 1977; Yanai, 1994) that combines plant and soil characteristics. By excluding effects of P depletion, the first calculation method (M1) provides the upper limit of P uptake potential. This approach illustrates the efficiency of nutrient capture (E) over time due to age-specific changes in root physiological characteristics, without soil properties as a factor influencing uptake. The second calculation method (M2) is used to illustrate the effect of different rates of soil depletion of P on the efficiency of nutrient capture (E) over time. The third calculation method (M3) simulates the field situation based on the estimated P availability at the root surface due to transport by diffusion and mass flow. The steady-state model (Yanai, 1994) requires a wide range of plant and soil characteristics as model inputs, most of which we were able to measure for our specific plants and soils (Table 1). Where estimates of parameters from the literature were required, most of the data were derived on the same soils that we used in our study (e.g. Wolf, 1988; Eissenstat & Yanai, 1997, Penn State University Soil Characterization Database System). Our simulation did not however, include phosphorus input into soil solution from sources such as fertilization, decomposition, or weathering. Moreover, we did also not represent P uptake by mycorrhizal hyphae, due to lack of data. Previous investigations by Williams & Yanai (1996) indicated that C_{av} and I_{max} are the most important variables explaining variation in nutrient uptake per unit root length across the parameter space defined by the ranges of values reported in the literature.

Parameters for calculating carbon costs

In the model, the cost term is defined as the carbon contained in the root plus that expended in growth and maintenance respiration since the birth of the root (Yanai *et al.*, 1995; Eissenstat & Yanai, 1997). Because construction costs probably contribute to the root respiration rates measured on the youngest classes of excised roots, we calculated the cost term as:

$$COST_{daily} = R_{observed} \times RQ \quad \text{Eqn 4}$$

$$COST_{cumlative} = C_{root} + \Sigma\,(COST_{daily}) \quad \text{Eqn 5}$$

($COST_{daily}$, the carbon cost on a daily basis (mmol C g^{-1} d^{-1}); $R_{observed}$, the respiration rate measured on excised root segments of known age (mmol O_2 g^{-1} d^{-1}); RQ, the respiratory coefficient (mol CO_2 [mol O_2]$^{-1}$); $COST_{cumlative}$, the carbon cost accumulated over the lifetime of a root (mmol C g^{-1}); and C_{root}, the carbon content of the root (mol C [g root]$^{-1}$).) Combining equations 3 and 5 shows that $E_{lifetime}$ is sensitive to the carbon content only while roots are young (i.e. when the value of $\Sigma\,(COST_{daily})$ is still small).

Results

Observations of root properties

Apple and citrus have very different root systems. Apple roots had a much shorter median lifespan (30 d) than did citrus roots (300 d) (Eissenstat *et al.*, 2000; Fig. 1). Apple had a much lower root length density than citrus, and much higher

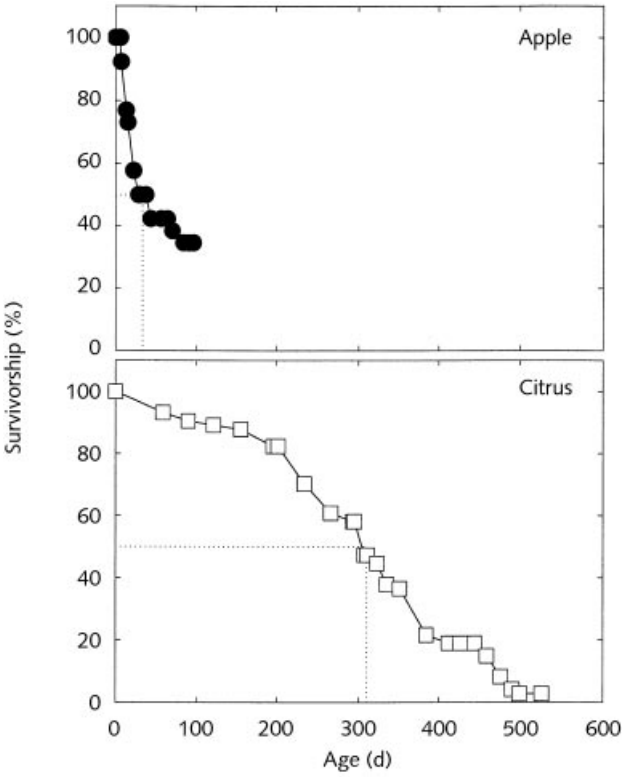

Fig. 1 Survivorship of roots from mature apple (Red Chief Delicious on M26 rootstock) and citrus (red grapefruit trees on sour orange rootstock) trees, measured using minirhizotrons (redrawn from Eissenstat *et al.*, 2000). Dotted lines indicate median root life spans.

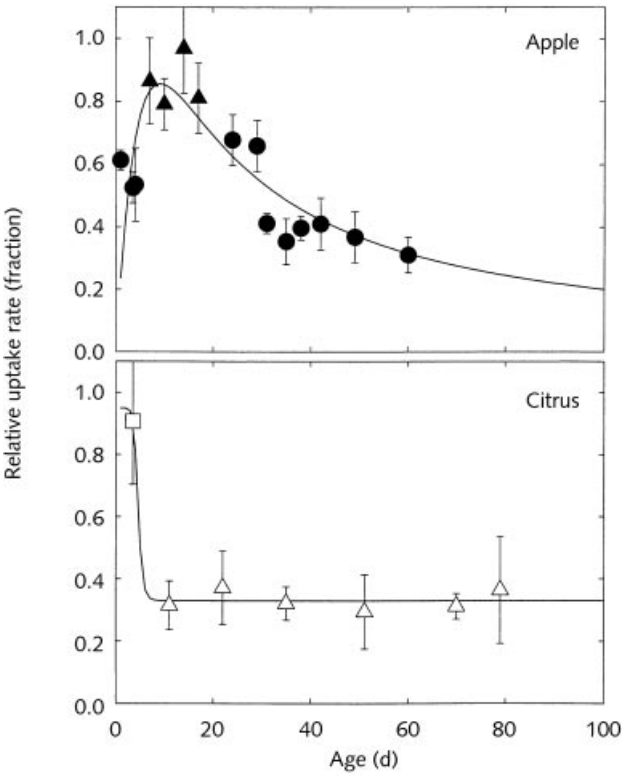

Fig. 2 Relative rates of P uptake (U_R, 'unitless') (± SE) as function of root age (A, d). Uptake of ^{32}P was measured over a range of concentrations from 1 to 1000 μM, using 1-cm excised root segments from mature trees. Data for apple (closed symbols; $r^2 = 0.60$) were fitted by $U_R = A \times (0.046 \times A^2 + 0.32 \times A + 3.85)^{-1}$. Data for citrus (open symbols; $r^2 = 0.98$) were fitted by $U_R = 0.33 + 0.62 \times (1 - [A^{10} \times (A^{10} + 4.54^{10})^{-1}])$. Triangles indicate the age classes that were used to analyse uptake kinetics (Fig. 3).

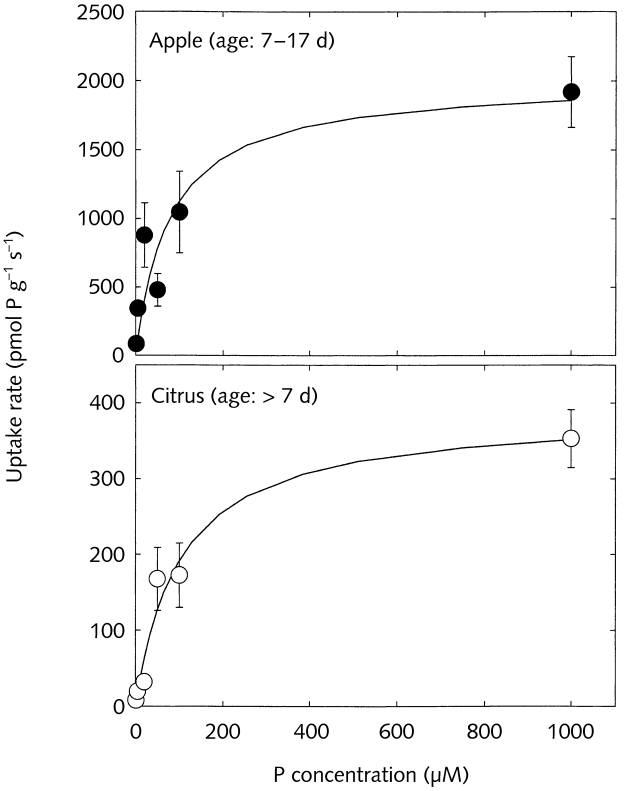

Fig. 3 Uptake rates (U_A, pmol P g^{-1} s^{-1}) (± SE) as a function of the concentration of P in solution ([P], μM). Average uptake rates were calculated by pooling roots aged between 7 and 17 d for apple and > 7 d old for citrus (marked with triangles in Fig. 2). Data were fitted to the equation: $U_A = I_{max} \times [P] \times (K_m + [P])^{-1}$, where I_{max} = 2001 pmol P g^{-1} s^{-1} and K_m = 77 μM for apple (closed symbols; $r^2 = 0.86$) and I_{max} = 388 pmol P g^{-1} s^{-1} and K_m = 102 μM for citrus (open symbols; $r^2 = 0.97$), respectively.

rates of water uptake per unit root length (Table 1). The maximum rate of P uptake (i.e. combining Figs 2 and 3) was also much higher in apple (2000 pmol g^{-1} s^{-1}) than in citrus (1100 pmol g^{-1} s^{-1}).

Both uptake characteristics and respiration rates varied strongly with root age. Apple roots took about 14 d to achieve peak rates of nutrient uptake (Fig. 2a). They maintained relatively high uptake rates (about 70% of the maximum) for a period of about 25 d. In contrast, the uptake capacity of citrus roots was highest in the youngest roots observed (4 d old), dropped within 1 wk to *c.* 35% of the maximum rate, and then exhibited little further decline with age (Fig. 2b).

Respiration rates were similar between the two species, except in very young roots (Fig. 4). In apple, root respiration rates decreased gradually with increasing age. In citrus, however, the youngest roots had very high respiration rates. The pattern of root activity with age was similar for P uptake and respiration in citrus but not in apple. We did not find a clear relation between root age and root C or N content in apple; for citrus we did not have age specific information on C and N content. The N content was on average 2.04 ± 0.06% (n = 39) for apple and 1.99 ± 0.06% (n = 28) for citrus; C contents are listed in Table 1.

Modelling efficiency of nutrient capture and root longevity

We simulated the daily and cumulative carbon costs and nutrient gains by apple and citrus roots by three different methods, using the observed patterns in uptake capacity and respiration as a function of root age. If uptake rates were assumed to equal I_{max}(M1), then both the daily and lifetime efficiency of nutrient capture continued to increase with root age (Figs 5 and 6), suggesting that roots should be retained indefinitely. That is, if nutrient uptake were limited only by physiological characteristics, efficiency of nutrient capture would be always improved by maintaining roots rather than replacing them. We also simulated a linear decrease in P availability (M2) by decreasing uptake rates from the maximum to zero over a period of 50 or 100 d. In these cases, the daily and

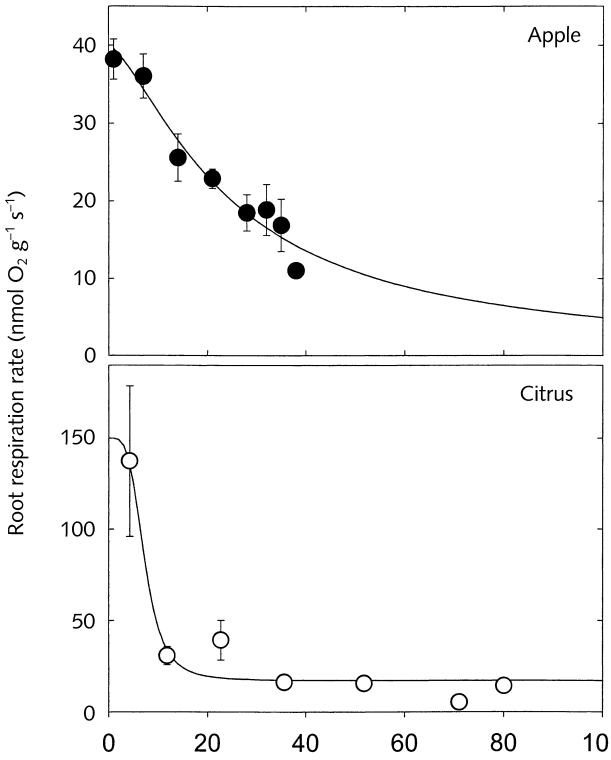

Fig. 4 Respiration rates (R, nmol O_2 g^{-1} s^{-1}) (± SE) as function of root age (A, d). Respiration was measured on 1-cm excised root segments from mature trees, using a Clark-type electrode. Data were fitted to the equation: $R = a - (a - b) \times (1 - [A^d \times (A^d + c^d)^{-1}])$, where a = 0, b = 40, c = 25.1 and d = 1.42 for apple (closed symbols; $r^2 = 0.95$) and a = 17.2, b = 150, c = 7.20 and d = 3.98 for citrus (open symbols; $r^2 = 0.48$), respectively. The values of a and b represent the minimal and initial respiration rates (nmol O_2 g^{-1} s^{-1}), respectively.

lifetime efficiencies do reach maximum values, with the optimal life span depending on the imposed rate of P depletion (Figs 5 and 6). This example illustrates the importance of estimating realistic rates of nutrient depletion, because of their influence on the simulated efficiency of nutrient capture.

We simulated depletion of soil P over time by assuming a closed system (M3), with no P inputs from fertilization, decomposition, or weathering. For citrus in the Florida soil, the daily and lifetime efficiency reached a maximum after 33 and 106 d (Fig. 6), respectively, which is much earlier than the observed median life span (300 d). Because of fertilization approximately every 90 d, the actual rate of soil depletion of P is probably intermediate between the extremes we simulated of infinite supply (M1) and zero supply (M3). Apple, with its sparse root system, did not deplete soil P stores enough to create a downturn in efficiency in our simulations in either soil (Fig. 5). Most likely, P is not the limiting soil resource for these apple trees, because of the very high buffering capacity of the Pennsylvania soil. The short life span of apple roots could be optimal for the acquisition of N, but we do not have the uptake parameters to test the efficiency of N uptake.

Discussion

Experimental observations

Understanding the fundamental relationship of root physiology to root age is essential in interpreting tradeoffs between maintaining existing roots and shedding and regrowing roots in more favourable soil locations. To our knowledge, the combined relationships of root respiration and P uptake capacity to root age have never before been reported for mature trees. Our results on two contrasting species, apple and citrus, clearly show that root age has a strong effect on root function and that this effect may vary among physiological characteristics (Figs 2 and 4). We expect age-related effects to be common in other species as well, with the shape of the age-response curves depending on species and environmental conditions. Despite the importance of root turnover in understanding ecosystem dynamics and individual plant success, we have only a rudimentary understanding of how the ageing process affects the ability of roots to provide nutrients for a given amount of photosynthate and how such measures of efficiency may be linked to root longevity. Depending on the ecosystem, other factors affecting root costs and benefits, such as mycorrhizal fungi, also should be assessed to more fully understand shifts in root efficiency with root age.

Our results allow us to make some unique observations on the relative costs of root construction and root maintenance. It takes only 12 d in apple, and 3 d in citrus, before the carbon used in root respiration (the cumulative daily C costs in Figs 5 and 6) exceeds the C content of the root (Table 1). If we assume that all respiration during wk 1 is growth respiration, that growth respiration plus root C content estimates root construction costs and that respiration after 1 wk is maintenance respiration, then it takes 30 d in apple and 62 d in citrus before the carbon used in maintenance respiration exceeds that used in root construction. Thus, the below-ground C expended for root maintenance may be at least as large as that expended for root construction in these two species. The only other work that has made fairly detailed estimates of the relative costs of root maintenance and construction as a function of age is in desert succulents where it takes about 90 d for root maintenance respiration to equal construction costs (Nobel *et al.*, 1992).

Newly formed apple roots had a low uptake rate for a few days although the underlying mechanisms are not clear (Fig. 2). Many of the root tissues may not be fully mature in roots this young, including immature xylem and phloem, endodermis and exodermis. In addition, a lag time before sufficient enzymes are produced to reach maximum enzyme capacity has been noted; however, these times are typically much shorter (*c.* 60 min in barley, Siebrecht *et al.*, 1995) than observed here.

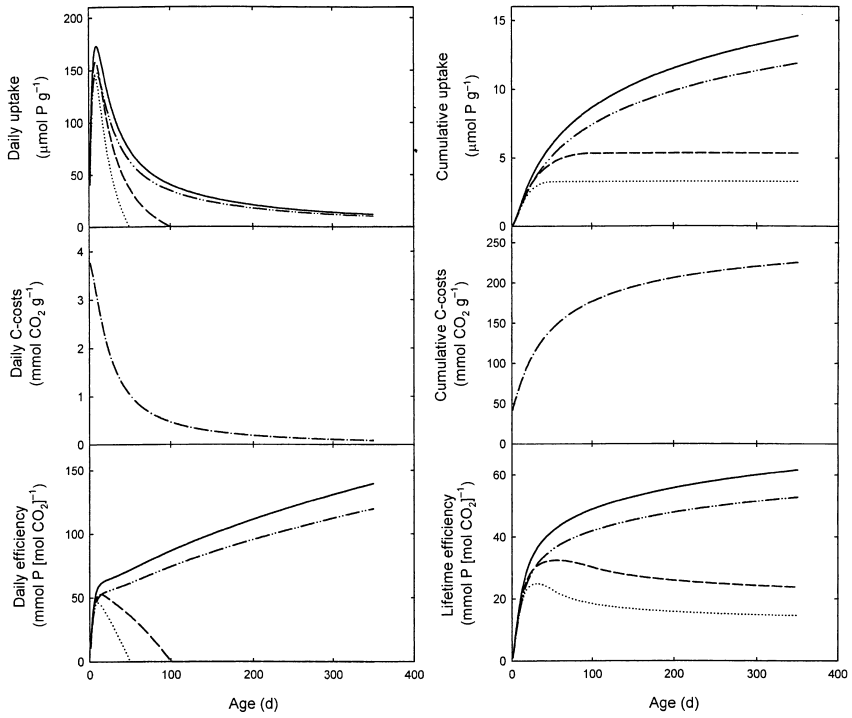

Fig. 5 Daily and lifetime efficiency (mmol P $[mol\ CO_2]^{-1}$) of apple roots, based on C costs and P uptake. C costs (dashed line with 1 dot) (mmol $CO_2\ g^{-1}$) were calculated by multiplying the respiration rate (nmol $O_2\ g^{-1}\ s^{-1}$) as a function of root age (Fig. 4) with a respiratory coefficient (RQ) of 1.1 mol CO_2 $[mol\ O_2]^{-1}$, and integrating over time with a time step of 1 d. Cumulative C-costs at age = 0 is assumed to be equal to the carbon content of the root; at age = 1 the respiratory costs of d 1 are included. The calculation of P uptake (μmol P g^{-1}) was based on the assumption that I_{max} (pmol P $g^{-1}\ s^{-1}$), which was determined for a single age class (Fig. 3), changed with root age according to the curve fitted for the relative uptake rate (Fig. 2). The K_m (μM) was assumed to remain constant. The P supply was either assumed to be not limiting (M1, solid line), assumed to be depleted in 50 or 100 d (M2, dotted line (50 d); dashed line (100 d)), or calculated using a steady-state model of solute uptake (M3, dashed line with two dots) (Nye & Tinker, 1977; Yanai, 1994). Lifetime P uptake was obtained by integrating over time with a time step of 1 d.

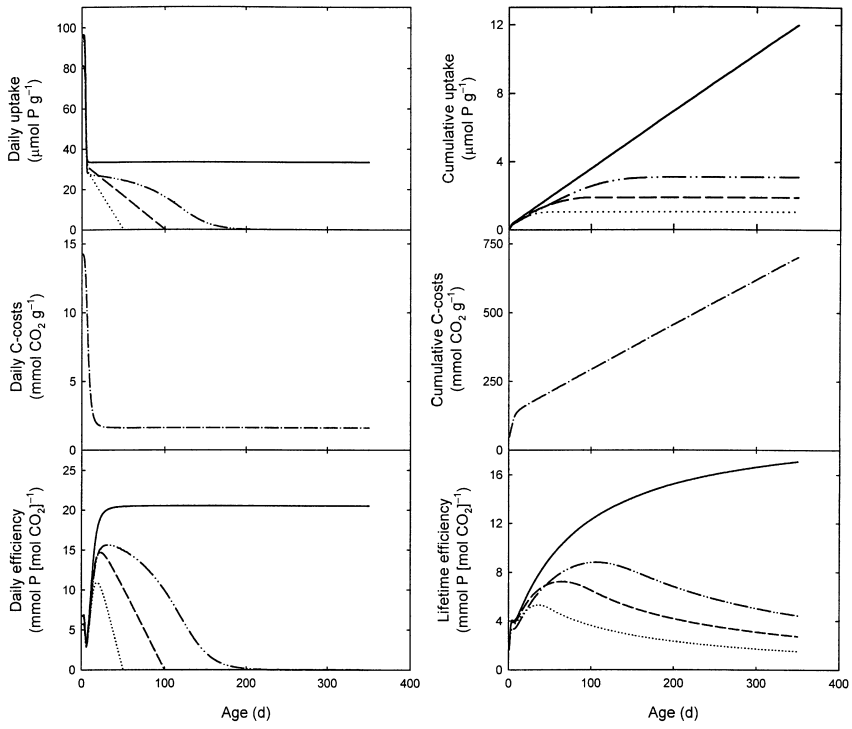

Fig. 6 Daily and lifetime efficiency (mmol P $[mol\ CO_2]^{-1}$) of citrus roots, based on the C-costs (mmol $CO_2\ g^{-1}$) and P uptake (μmol P g^{-1}). Details are as described for apple in Fig. 5. C costs (dashed line with 1 dot) (mmol $CO_2\ g^{-1}$) were calculated by multiplying the respiration rate (nmol $O_2\ g^{-1}\ s^{-1}$) as a function of root age (Fig. 4) with a respiratory coefficient (RQ) of 1.1 mol CO_2 $[mol\ O_2]^{-1}$, and integrating over time with a time step of 1 d. Cumulative C-costs at age = 0 is assumed to be equal to the carbon content of the root; at age = 1 the respiratory costs of d 1 are included. The calculation of P uptake (μmol P g^{-1}) was based on the assumption that I_{max} (pmol P $g^{-1}\ s^{-1}$), which was determined for a single age class (Fig. 3), changed with root age according to the curve fitted for the relative uptake rate (Fig. 2). The K_m (μM) was assumed to remain constant. The P supply was either assumed to be not limiting (M1, solid line), assumed to be depleted in 50 or 100 d (M2, dotted line (50 d); dashed line (100 d)), or calculated using a steady-state model of solute uptake (M3, dashed line with two dots) (Nye & Tinker, 1977; Yanai, 1994). Lifetime P uptake was obtained by integrating over time with a time step of 1 d.

In various species, nutrient uptake capacity and radial transport at different positions along root axes have been studied by exposing sequential root segments to labelled nutrients (Clarkson, 1991). These studies show that ion transport capability is widely distributed over the root surface, and not restricted to the apical zones. Uptake capacity may be highest in the youngest parts of the root axis where the effective root surface can be described by the perimeter of the tip of the root hairs; older root hairs further down the root are mostly not functional. Secondary deposition of suberin lamellae and development of a tertiary wall in the endodermis can restrict the movement of ions into the xylem. These generalizations,

however, are based mostly on studies of seminal roots of agricultural herbaceous species (Clarkson, 1991). In general, studies that use the distance along the axis to obtain contrasting root ages in agricultural species reflect smaller age differences than we studied, making direct comparison impossible. Moreover, the seminal (indeterminate) roots of agricultural herbaceous species may be inherently different from the fine, ephemeral (determinate) roots of mature trees. In general, a diminishing uptake capacity with increasing root age appears to be a useful adaptation, as the soil surrounding older roots is likely to be more depleted than the soil surrounding a newly produced root (Jungk, 1991).

The declining respiratory costs we observed with increasing root age are consistent with reduced uptake capacity or enzymatic activity in ageing roots. For example, in woody species, lower N concentrations, which presumably are correlated with enzyme capacity, are associated with lower respiration costs (Pregitzer *et al.*, 1998). Similarly, maintenance costs were lower for nonactive citrus roots in droughted soil patches than for active roots in irrigated soil patches (Bouma *et al.*, 2000). However, in apple we found that the N content ($2.04 \pm 0.06\%$) was independent of root age. The decrease in root respiration with increasing age in apple was very similar to that observed in grape (Comas *et al.*, 2000). In cactus species, older roots had lower respiration rates (Palta & Nobel, 1989a,b). Roots that differ in diameter also differ in physiology and longevity (Pregitzer *et al.*, 1998; Wells & Eissenstat, 2001), analogous to the physiological differences of roots of contrasting ages. In general, the youngest and the finest roots tend to be the most active. Much can still be learned about root physiology by studying well-defined classes of roots as opposed to average responses of populations of roots.

Excision of the roots was the only method available that allowed us to relate respiration and uptake capacity to root age. The use of excised roots may have biased the absolute values of the rates of root respiration and nutrient uptake, but we believe that the patterns we observed as a function of root age (Figs 2 and 4) reflect the behaviour of intact roots, and provide useful input to the simulations (Figs 5 and 6). Fitting uptake kinetics was difficult, because we had only few observations at high P concentrations. The total number of samples needed to distinguish the effect of root age (approximately 80 for citrus and 180 for apple) limited the number of P concentrations we could use in our uptake studies. If there is an error in our estimates of the absolute values of Km and Imax, such error will not affect the pattern of uptake with root age (Fig. 2), which is most important for simulating the age where roots maximize their lifetime efficiency.

Efficiency of nutrient capture as a predictor of root longevity

It has been difficult in the past, without age-dependent functions for root respiration and uptake rates, to estimate the theoretically optimal root life span (Yanai *et al.*, 1995; Eissenstat & Yanai, 1997). Having established such age-dependent functions on two widely contrasting species, apple and citrus, we explored the age at which roots maximize the efficiency of nutrient capture and whether the root life span that maximizes this efficiency bears any relation to the observed life span.

Besides our simulations with the steady-state model (M3) that uses realistic inputs for citrus in sandy soil and apple in silt loam soil, we examined root efficiency in hypothetical soils where efficiency was either not soil limited (M1) or where soil nutrients were depleted at a constant rate for both plants (M2). One of the surprising findings of our simulations is that soil characteristics are as important as age-dependent root characteristics in determining the age at which the efficiency of nutrient capture is maximized (Figs 5 and 6). If nutrient supply to the root was not limiting (M1), we found that the efficiency of nutrient capture continued to increase throughout the simulation period of 350 d, despite the decrease in root activity with increasing root age for both apple and citrus. If the soil nutrient pool was depleted over time by simulating nutrient uptake (M3), then citrus in Florida soil (Fig. 6) reached a maximum for the lifetime efficiency of nutrient capture at 106 d, suggesting that roots should not live as long as the observed median lifespan of 300 d. However, fertilization was not included in our simulation, whereas in reality the citrus trees in our field site are fertilized four times a year. In addition, P acquisition by mycorrhizal hyphae was not included in these simulations. Mycorrhizal hyphae may acquire P from soil solution outside the root depletion zone, which can be very important for P uptake by citrus as the soil becomes depleted in P (e.g. Eissenstat *et al.*, 1993). Theoretical explorations have emphasized the efficiency of mycorrhizal hyphae based on their very small diameter (Yanai *et al.*, 1995). A more complete analysis of the efficiency of mycorrhizal fungi over the lifetime of a root must wait until it is possible to quantify key components of mycorrhizal root efficiency, including the longevity, uptake capacity and respiration of extramatrical hyphae over the lifetime of the root. Including any factor that increases root P acquisition in older roots (e.g. by lengthening the period for depletion due to fertilization or mycorrhizal-mediated P uptake) would have resulted in an increase and thus more realistic prediction of the root life span in citrus. Our results suggest, moreover, that changes in nutrient uptake rather than changes in carbon costs are more important at affecting the age at which the lifetime efficiency of nutrient capture is maximized (and a root should be shed).

The hypothesis that root longevity maximizes the efficiency of nutrient capture assumes that plants control root death. Observations of increased root longevity upon water and nutrient addition in mixed hardwoods (Pregitzer *et al.*, 1993; Fahey & Hughes, 1994) support the theory that root mortality is controlled by feedback mechanisms, but this response may not be universal (Gross *et al.*, 1993). Alternatively, root

longevity may be to some extent controlled by external factors such as soil microorganisms. Several observations support this hypothesis. For example, in years with high densities of the pathogenic fungus, *Phytophthora nicotiana*, root longevity of citrus was about 2 wk shorter (Kosola *et al.*, 1995) than in years with lower densities of *Phytophthora* (Eissenstat & Yanai, 1997). The median life span of sugar maple (*Acer saccharum*) roots (40 wk) was prolonged by the addition of fungicide (to 99 wk) and by the addition of insecticide in combination with fungicide (> 101 wk; Wells, 1999; Eissenstat *et al.*, 2000). It remains possible that the plant influences the rate of root loss due to herbivory and pathogens through the production of defensive compounds or inert tissues. For example, root browning following severe nematode exposure has been observed in apple (Gunn, 1979), which may reflect the local accumulation of defensive compounds such as phenolics (McKenzie & Peterson, 1995). There is also a strong relationship between root longevity and tissue density (Ryser, 1996). However, we still lack the quantitative understanding that would allow us to model the effect of herbivory and pathogens on root longevity (Eissenstat & Yanai, 2001). These effects are made more complex by interactions among the soil biota. For example, infection with mycorrhizal fungi can reduce damage to the roots by other soil microbes (Grange *et al.*, 1994; Newsham *et al.*, 1995).

Conclusions

We showed a species-specific effect of root age on the respiration and P uptake capacity of apple and citrus roots. These data are unique in that they include costs (respiration) and benefits (uptake) in the same study, and they used the fine, ephemeral (determinate) roots of mature trees, which are very different from the more commonly studied indeterminate (seminal) roots of agricultural herbaceous species. By using our measurements as input in simulations of daily and lifetime efficiency, we were able to demonstrate that soil characteristics are as important as age-dependent root characteristics in determining the optimal life span, at which the lifetime efficiency of nutrient capture is maximized. We also concluded that the optimal life span is likely to be more dependent on changes in nutrient uptake than carbon costs, which are unlikely to remain high in an older root with reduced uptake. Future studies should aim at including the effects of mycorrhizal fungi, herbivores, pathogens, and root defense on age-related nutrient uptake, carbon costs, and root efficiency.

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