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Topsoil foraging and phosphorus acquisition efficiency in maize (*Zea mays*)

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Abstract. In soybean and common bean, enhanced topsoil foraging permitted by shallow root architectures is advantageous for phosphorus acquisition from stratified soils. The importance of this phenomenon in graminaceous crops, which have different root architecture and morphology from legumes, is unclear. In this study we evaluated the importance of shallow roots for phosphorus acquisition in maize (*Zea mays* L.). In a field study, maize genotypes with shallower roots had greater growth in low phosphorus soil than deep-rooted genotypes. For physiological analysis, four maize genotypes differing in root shallowness in the field were grown in solid media with stratified phosphorus availability in a controlled environment. Of the four genotypes, one shallow and one deep genotype were also inoculated with arbuscular mycorrhiza (AM). Shallower genotypes had significantly greater growth and phosphorus accumulation compared with deeper genotypes at low phosphorus availability. Mycorrhizal colonisation altered root shallowness under low phosphorus conditions, increasing shallowness substantially in a deep-rooted genotype but slightly decreasing shallowness in a shallow-rooted genotype. Mycorrhizal colonisation increased phosphorus acquisition under low phosphorus availability. Respiration costs of roots and shoots of phosphorus-efficient genotypes were significantly lower under low phosphorus conditions compared with inefficient genotypes. The physiological efficiency of phosphorus acquisition, expressed as root respiration per unit of phosphorus acquisition, was greater in shallow rooted genotypes. Our results demonstrate that genetic variation for root shallowness exists in maize, that phosphorus and AM can modulate root shallowness independently, and that a shallower root system is beneficial for plant performance in maize at low phosphorus availability. We propose that root architectural traits that enhance topsoil foraging are important traits for improved phosphorus acquisition efficiency of annual grain crops such as maize in addition to legumes.

Keywords: phosphorus efficiency, respiration, root cost–benefit analysis, root shallowness, vesicular-arbuscular mycorrhizae, *Zea mays*.

Introduction

Low soil phosphorus availability is a primary constraint for plant growth in many terrestrial ecosystems (Lynch and Deikman 1998). Accordingly, plants have evolved a wide array of adaptations to enhance phosphorus acquisition from soils (Schachtman *et al*. 1998; Raghothama 1999; Vance *et al*. 2003; Hammond *et al*. 2004). One set of adaptive responses is the alteration of root architecture to increase phosphorus acquisition from the soil at minimum metabolic cost (Lynch and Brown 2001; Lynch and Ho 2005). Root architecture, defined as the spatial configuration of a root system (Fitter 1991; Lynch 1995), is particularly important for phosphorus acquisition because of the relative immobility of phosphorus in soils (Sample *et al*. 1980; Barber 1995). In agricultural ecosystems, the limited access of farmers in developing countries to mineral phosphate fertilisers and the excessive runoff and erosion from phosphate fertilisation in industrialised countries, have inspired interest in developing crop genotypes that require less phosphorus fertiliser (Sanchez 1976; Lynch 1998). For example, our research with common bean has demonstrated the utility of root architectural traits to breed

Abbreviations used: AM, arbuscular mycorrhizae; AMIR, arbuscular mycorrhizae infection rate; DAP, days after planting; IRR, intact root respiration; NCG, net C gain; PAE, phosphorus acquisition efficiency; RGR, relative growth rate; RPC, root phosphorus content; RPCO, root phosphorus concentration; RRL, relative root length; SPAR, specific phosphorus absorption rate; SPC, shoot phosphorus content; SPCO, shoot phosphorus concentration; TRL, total root length.

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for enhanced phosphorus acquisition efficiency (PAE) (Lynch and Beebe 1995; Bonser *et al*. 1996; Lynch and Brown 1998; Liao *et al*. 2001, 2004).

Maize is generally considered to have a high fertility requirement, but variation has been known to exist among maize genotypes for phosphorus efficiency for more than a century (Anonymous 1887; Clark and Brown 1974; Naismith *et al*. 1974; Nielsen and Barber 1978; DaSilva and Gabelman 1993; Gaume *et al*. 2001; Fan *et al*. 2003). However, attempts to improve phosphorus efficiency through field selection are difficult because of the complexities of soil phosphorus bioavailability and its interaction with many variables of the aboveground and belowground environments, as well as the interaction of multiple plant traits related to phosphorus acquisition and utilisation. Therefore, genetic improvement through selection for specific traits may be more fruitful than yield screening. To this end, a better understanding of physiological traits conferring phosphorus efficiency is needed.

Topsoil foraging permitted by architectural traits that deploy roots to surface horizons is advantageous for phosphorus acquisition in many soils (Lynch and Brown 2001). In most natural soils, phosphorus availability is greater in surface or near-surface horizons than in the subsoil, principally because of the continual deposition of phosphorus onto the soil surface in senesced leaves and other plant residues. In agricultural soils, fertilisation and cultivation increases phosphorus in the topsoil, with only very slow movement of phosphorus into the subsoil. As a result, phosphorus availability is usually greatest in the topsoil and declines substantially with soil depth (Chu and Chang 1966; Enwezor and Moore 1966; Keter and Ahn 1986). Geometric modelling indicates that shallower root systems are inherently more efficient in acquiring phosphorus because of co-localisation of soil nutrient availability and root activity, and less inter-root competition (Ge *et al*. 2000). Geometric modelling also indicates that shallower root systems are more competitive with neighbouring plants for topsoil nutrients (Rubio *et al*. 2001). This result has been confirmed in the field (Rubio *et al*. 2003). In common bean, phosphorus availability regulates basal root shallowness in some genotypes, and genotypic adaptation to low phosphorus availability is associated with the ability to allocate roots to shallow soil horizons under low phosphorus availability (Bonser *et al*. 1996; Liao *et al*. 2001, 2004).

Phosphorus-deficient plants generally exhibit retarded growth and increased root : shoot ratio (Atkinson 1973; Lynch *et al*. 1991; Mollier and Pellerin 1999). The decrease in shoot growth of phosphorus-deficient plants is directly caused by a reduction of leaf expansion and reduced leaf initiation (Lynch *et al*. 1991), possibly led by decreased root hydraulic conductance (Radin and Eidenbrock 1984) mediated by aquaporins (Clarkson *et al*. 2000) and reduced transfer of cytokinins from root to shoot (Salama and Wareing 1979). Allocation of plant carbon to roots for the purpose of acquiring more phosphorus is of obvious importance for adaptation to low phosphorus availability. In common bean, low phosphorus availability significantly increases the proportion of assimilated carbon devoted to root growth and maintenance (Nielsen *et al*. 1998, 2001). Moreover, phosphorus-efficient genotypes have lower rates of root respiration than inefficient genotypes, and as a result are able to sustain greater root growth than inefficient genotypes (Nielsen *et al*. 2001). It is evident that carbon economy is important for plant survival and success under phosphorus stress (Lynch and Ho 2005).

Arbuscular mycorrhizas enhance phosphorus uptake resulting in increased plant growth, particularly when phosphorus availability is low (Clarkson 1985; Rubio *et al*. 2002). This may result from a greater absorbing surface area and absorbing length by hyphae (Cooper 1984), exploration of soil beyond the zone of diffusion-driven phosphorus depletion around the root (Li *et al*. 1991), and higher phosphorus-use efficiency (Koide 1991). Mycorrhizas have also been shown to alter root architecture. In some cases AM has been shown to increase branching (Norman *et al*. 1996), while in others it has been shown to reduce the relative amount of root branching, and to affect root morphology and topology by changing the specific root length (root length per unit of root weight) and the number and diameter of higher order roots (Hetrick *et al*. 1988). These changes were especially evident in low fertility soils. Despite affecting overall phosphorus acquisition and certain aspects of root architecture, mycorrhiza may not actually affect the ranking of different genotypes in terms of biomass production under low phosphorus conditions, as in the case of common bean (Lynch and Beebe 1995; Yan *et al*. 1995).

The benefits of AM association do not come without a cost to the plant. AM plants have been found to have increased translocation of photosynthate to root systems in many species (Baas *et al*. 1989; Jakobsen and Rosedahl 1990). Baas *et al*. (1989) found in *Plantago major* L. that root respiration per unit leaf area increased by 20–30% for AM plants. The total respiratory costs of mycorrhizal symbiosis are a considerable component of the overall carbon economy of the host plant, and consumes between 2 and 17% of daily photosynthate, varying among plant and fungal species, and plant age and mycorrhizal development (Bryla and Eissenstat 2005). It is also affected by environmental conditions such as soil nutrient availability, soil temperature and moisture, light conditions, elevated atmospheric CO_2 and ozone pollution (Bryla and Eissenstat 2005). So while AM infection can result in increased relative growth rates due to increased phosphorus acquisition, the benefits of the fungal symbiosis come at a considerable carbon cost to the plant and may be worthwhile only when phosphorus rather than carbon is the primary limiting factor for growth. In common bean, mycorrhizal infection in low phosphorus plants increased the

root specific phosphorus absorption rate, but a concurrent 11–15% increase in root respiration consumed the increased net C gain resulting from greater phosphorus uptake (Nielsen *et al*. 1998). It has been proposed that the increase in root respiration in mycorrhizal roots was mainly due to increased maintenance and growth respiration of the fungal tissue (Nielsen *et al*. 1998).

The deployment of root architectural traits in plant breeding programs has great potential to alleviate a primary constraint to crop production in world agriculture. Recent advances with legumes need to be confirmed with cereals, which have distinct root morphology and architecture. The objective of the present study was to assess the value of topsoil foraging for phosphorus acquisition in maize, including an examination of the effects of mycorrhizal colonisation on root shallowness, and physiological analysis of the effects of mycorrhizal colonisation and root shallowness on phosphorus acquisition efficiency.

Materials and methods

Plant materials / field study

Maize (*Zea mays* L.) seeds of four F1 genotypes, H99 × W64A, B73 × WH, Oh43 × B37, and H99 × Oh43, were planted at the University of Wisconsin Arlington Agricultural Research Station near Arlington, WI (43°8′N, 89°20′W) in 2001 and 2002. The soil was a Withee silt loam (fine-loamy, mixed, mesic Aquic Glossoboralf). There were two biological replicates for each genotype per year, and each replicate had 60 plants grown in two 6.1-m rows. The low phosphorus soil had 16 ppm available phosphorus, the soil pH was 6.3 with 2.6% of organic matter. Two representative root crowns of 7-week-old seedlings in each replicate were excavated, soaked in a container filled with water for 1 h, rinsed with a fine spray of pressurised water, and collected on a sieve in early July 2001 and 2002. The intact root image of each plant was scanned into a computer by image-analysis software (Delta-T SCAN, Delta-T Devices Ltd, Cambridge, UK). Root length at different horizontal layers was separated and collected by this software. Relative root length in the upper 5-cm horizontal layer was determined by dividing root length of the upper 5-cm horizontal layer over the total root length. The arithmetic mean ($n = 4$) was used for correlation analysis. In preliminary studies, H99 × W64A and B73 × WH genotypes had been characterised as being phosphorus inefficient with deep root systems, yet responsive to phosphorus fertilisation; while Oh43 × B37 and H99 × Oh43 genotypes were characterised as being phosphorus efficient with shallow root systems, yet responsive to phosphorus fertilisation (Fig. 1). These four genotypes did not differ for root hair length in cigar roll culture before transplanting, which is important since genotypic variation in root hair length is associated with enhanced adaptation to low soil phosphorus availability in maize (Zhu 2003).

Greenhouse study

Experimental design

The greenhouse experiment was a 2 × 2 × 4 factorial design with two phosphorus treatments, one with uniformly high phosphorus availability with depth and one with greater phosphorus availability in the topsoil (as discussed below), two harvests (10 and 20 d after transplanting), four genotypes (H99 × W64A, B73 × WH, Oh43 × B37 and H99 × Oh43), and four replicates staggered 1 d between replicates. In addition, H99 × W64A and Oh43 × B37 were also inoculated with vesicular-arbuscular mycorrhizae, four replicates of each.

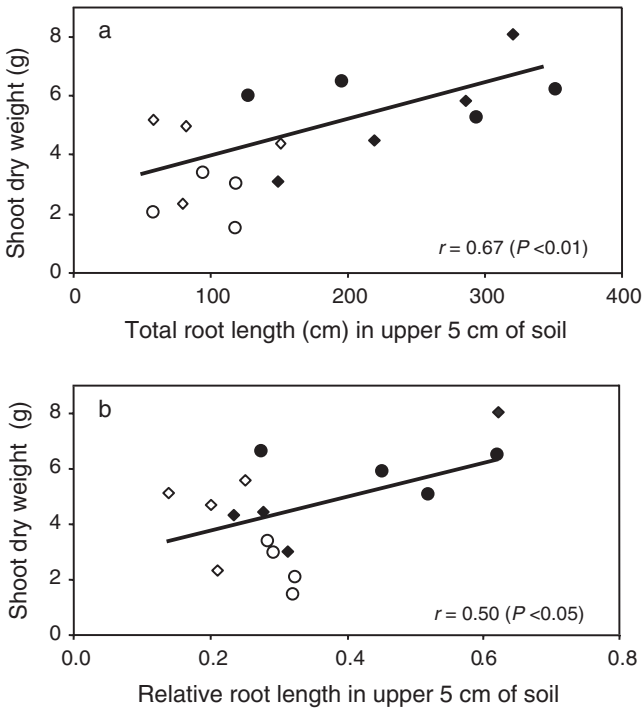

Fig. 1. Correlation (Pearson linear correlation coefficient, r, df = 14) between root shallowness (expressed as both absolute and relative root biomass in the topmost 5 cm of soil) and shoot biomass at 7 weeks after planting for four maize genotypes in a field with low phosphorus availability. ◆, Oh43 × B37; ●, H99 × Oh43; ◇, H99 × W64A; ○, B73 × WH.

Growth conditions

Uniform (representative) seeds of the four genotypes were selected, surface sterilised for 1 min in 0.5% solution of NaOCL, washed twice in deionised H_2O, and germinated. Seeds were placed in darkness at 28 ± 1°C for 2 d. Seedlings of similar size were transferred to 9.1-L (24 cm width × 20 cm height) plastic pots filled with 1 : 1 (v : v) of acid-rinsed solid-phase-buffered sand (Lynch *et al*. 1990) and medium-grade vermiculite. Each pot had a 2% weight / volume mixture of phosphorus-doped alumina (Lynch *et al*. 1990) and silica sand (99.808% SiO_2, Silica of Ottawa, Flint Shot, 41–43 mesh, Ottawa, IL). Fibreglass mesh (1.5 mm) was installed at 7-cm depth from the surface of the pot. Each pot was filled with media to 1 cm from the surface of the pot, making the upper layer 6 cm thick (1–7 cm depth) and the bottom layer 23 cm thick (7–30 cm depth). Preliminary studies showed that plant and root growth of maize was not adversely affected by the presence of the mesh. For the low phosphorus treatment, phosphorus availability was maintained at 5.2 μM in the upper layer and 0.8 μM in the lower layer of the pots. The high phosphorus treatment consisted of a constant availability of high (32.4 μM) phosphorus concentration throughout the pot. Sand substrate in the top 6 cm of inoculated pots was evenly mixed with vesicular-arbuscular mycorrhizae innoculum including *Glomus intraradices*, *Glomus aggregatum* and *Glomus mosseae* (AM80, Tree of Life Nursery and GroPower Inc. Chino, CA). The application rate of mycorrhizal inoculum was 2.5 g per pot. Twice daily (0700 and 1400 h), the pots were irrigated with nutrient solution consisting of (in μM): K (3000), NO_3 (7000), NH_4 (1000), Ca (2000), SO_4 (500), Mg (500), Cl (25), B (12.5), Mn (1), Zn (1), Cu (0.25) Mo (0.25) and EDTA–Fe (25). The plants were grown in a temperature-controlled greenhouse at University Park, PA (40°49′N, 77°49′W) with a photoperiod of 14 / 10 h

at 28 / 24°C (light / darkness). Maximum midday photosynthetic flux densities reached 1400 µmol photons m^{-2} s^{-1}. The relative humidity was 50% day and night. The nutrient solution pH was adjusted to 5.8 daily.

Gas-exchange measurements

Net canopy photosynthesis rate (µmol m^{-2} s^{-1}) was measured at day 19 after transplanting between 1100 and 1230 h with a portable infrared gas analyser (Li-Cor 6200, Li-Cor, Lincoln, NE). A 5-L mylar chamber was used to enclose the whole shoot and each measurement was completed in 1 min. The leaf area of each plant was determined by image-analysis software (Delta-T SCAN, Delta-T Devices Ltd). Shoot respiration rate was measured with a portable infrared gas analyser (Li-Cor 6200, Li-Cor) 2 h after sunset (2030–2130 h) on day 19 after transplanting. Numerical analysis was used to integrate shoot photosynthesis measurements into diurnal carbon budgets (Lynch and Rodriguez 1994). Daily shoot CO_2 assimilation rate was calculated as net daytime C assimilation minus night shoot respiration (Nielsen *et al*. 1998). For measurement of root respiration we used the 'head space' approach of sampling air flowing over the soil surface. Studies of common bean and citrus root respiration in soil (Bouma *et al*. 1997*a*, *b*) indicated that in such conditions root respiration was not influenced by soil moisture or soil CO_2 concentration, and there was no difference in intact root respiration when comparing 'head space' measurements with 'perfusive' measurements. At day 20 after transplanting, the root system was sealed off from the shoot with a PVC plate placed on top of the pot. An air pump provided a stable flow of air through the 'head space' compartment of the pot with a flow rate of 1200 µmol s^{-1}. The measurements were conducted in early morning (0600–0800 h) with a portable infrared gas analyser in a differential mode (Li-Cor 6250, Li-Cor), ensuring that the 'head space' CO_2 concentration remained ~900 µmol mol^{-1} and that the 'head space' temperature only increased slightly (<1.5°C). During the measurement, CO_2 changed less than 20 µmol mol^{-1}. Below-ground CO_2 generation due to decomposition of the AM inoculum was estimated in pots with inoculum but without plants. Root-derived respiration of plants with the inoculum was calculated as gross below-ground respiration minus respiration due to decomposition of inoculum.

Growth analysis

Before transplanting, the plant shoot and root dry weight and phosphorus concentration of 2-d-old seedlings were measured as a baseline. Plants were harvested at days 10 and 20 after transplanting. Shoots and roots were separated from the sand by submerging pots in a container filled with water. Intact roots were carefully collected and rinsed in deionised water. Roots from the soil horizons were harvested separately. Total root length from the upper and lower horizons was obtained by scanning with image analysis software (WinRhizo Pro, Régent Instruments, Québec, Canada). Relative root length in the upper horizon was calculated as the root length in the upper horizon divided by total root length of the plant. Shoots and roots were dried at 60°C for 72 h for biomass determination. Relative growth rate (RGR_{DW}; Hunt 1990) by numerical differentiation of the dry weight data was obtained from second degree, three-point formulae (Erickson 1976).

Phosphorus accumulation

Subsamples of the dried samples of roots and shoots were ground and ashed at 495°C for 10 h. The ash was dissolved in 4 mL of 100 mM HCl and analysed for phosphorus concentration spectrophotometrically (Murphy and Riley 1962). Specific phosphorus absorption rate (SPAR) is a measure of the net phosphorus absorption rate per unit root biomass (mg phosphorus g^{-1} root DW d^{-1}) over the interval t_1 to t_2.

$$SPAR = (M_2 - M_1)/(t_2 - t_1) \times (\lg R_2 - \lg R_1)/(R_2 - R_1),$$

where M is the phosphorus content per plant, and R is root biomass (g) (Hunt 1990).

Mycorrhizal colonisation

Mycorrhizal colonisation was evaluated on a random subsample of 25 root segments collected before drying the root system. Root pieces were placed in histocaps and were cleared for 15 min in 10% KOH at 121°C. The samples were then rinsed with water and incubated in 5% HCl overnight. Samples were then stained overnight with 0.05% trypan blue (Sigma, St. Louis, MO) in equal amounts of glycerol, lactic acid, and water. The following day, the root samples were destained in equal amounts of glycerol, lactic acid, and water (Phillips and Hayman 1970). Stained root samples were then examined under a microscope. The percentage of root length colonised is expressed as the numbers of intersections with root colonisation out of 100 total intersections counted in a grid intersect methodology (Tennant 1975).

Statistical analysis

Data were analysed with the Minitab statistical package (Minitab Inc. University Park, PA). Relative root length, total root length, plant dry weight, relative growth rate, root : shoot ratio, shoot and root phosphorus content, and specific phosphorus absorption rate were first subjected to ANOVA for main effects and first-order interactions using a general linear model that included phosphorus and genotype factors. Genotype, mycorrhizal status, and phosphorus availability were considered fixed effects, and replicates were random. Fisher's least significance difference was used for multiple comparisons under *post hoc* ANOVA. Analysis of covariance was performed for root shallowness using root dry weight as a covariant (Statview, SAS Inc. Cary, NC).

Results

Field study

Root shallowness was significantly correlated with shoot biomass under low phosphorus conditions in the field (Fig. 1). Of the four genotypes studied, two were relatively shallow and phosphorus efficient, while two were deeper and less phosphorus efficient. This correlation was stronger for absolute root length in shallow soil (Fig. 1*a*) than for relative root length in shallow soil (Fig. 1*b*), because of the allometric effects of plant size, but was significant for both absolute and relative measures of root shallowness.

Greenhouse study

Mycorrhizal colonisation

Mycorrhizal colonisation averaged 28% in inoculated plants in low phosphorus media, which was significantly greater than inoculated plants at high phosphorus, which had 15% colonisation ($P<0.05$). Mycorrhizal infection averaged <5% in non-inoculated plants.

Root shallowness

At high phosphorus, total root length was the same for all genotypes, although the relative root length in the topsoil was slightly greater for the phosphorus-efficient genotypes (Fig. 2). At low phosphorus and without mycorrhizal inoculation, the phosphorus-inefficient genotypes (H99 × W64A and B73 × WH) had significantly

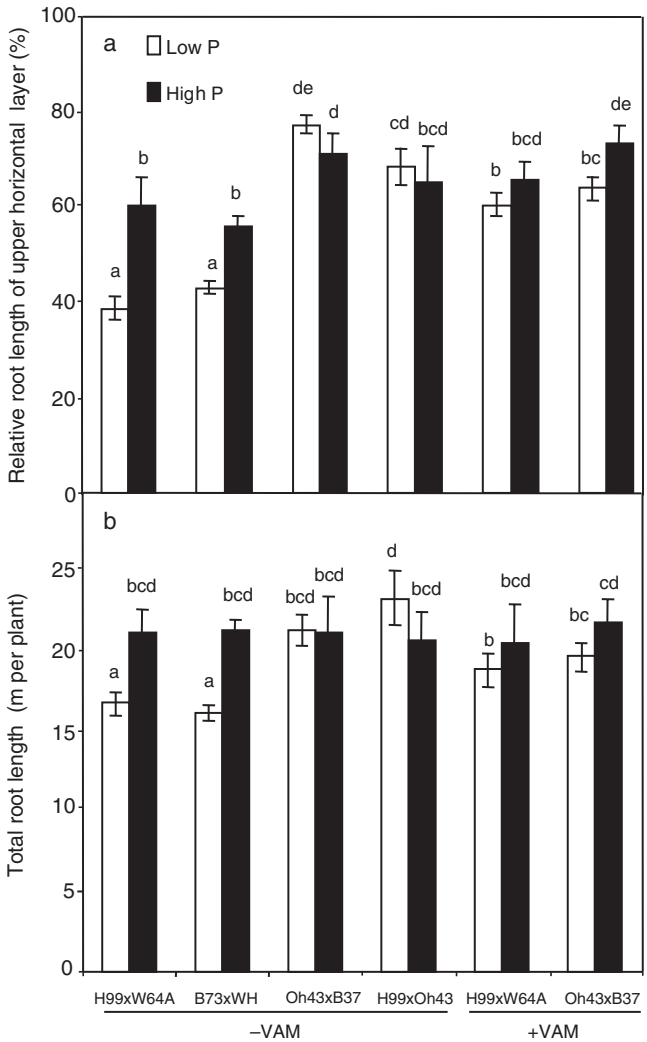

Fig. 2. Relative root length in the upper horizontal layer (*a*) and plant total root length (*b*) at 20 d after transplanting of four maize genotypes, as influenced by phosphorus availability in the growth media and mycorrhizal symbiosis. Relative root length was calculated as the percentage of root length in upper layer relative to total root length of a plant. Data shown are means ± SE of the mean ($n = 4$). Means with same letter are not significantly different ($P \leq 0.05$).

lower total and relative root length in the topsoil than efficient genotypes (Oh43 × B37 and H99 × Oh43) (Fig. 2*a*). Low phosphorus availability decreased total root length and relative root length in the topsoil for phosphorus-inefficient genotypes (H99 × W64A and B73 × WH), but not for phosphorus-efficient genotypes (Oh43 × B37 and H99 × Oh43) (Fig. 2). With mycorrhizal inoculation at low phosphorus, the phosphorus-inefficient genotype (H99 × W64A) increased root length in the topsoil, whereas the phosphorus-efficient genotype (Oh43 × B37) decreased root length in comparison with non-inoculated plants (Fig. 2).

Plant growth measurements

Low phosphorus availability reduced growth by ~41% across the four genotypes (Fig. 3). For non-inoculated plants, shallow genotypes had significantly greater biomass than deep genotypes at low phosphorus availability, while there was no difference among the four genotypes at high phosphorus availability (Fig. 3). Although plant growth was enhanced for AM-inoculated plants of genotypes H99 × W64A and Oh43 × B37 in low phosphorus media, the ranking of these two genotypes was independent of mycorrhizal inoculation (Fig. 3). The phosphorus-efficient genotype performed better than the phosphorus-inefficient genotype, regardless of the presence of

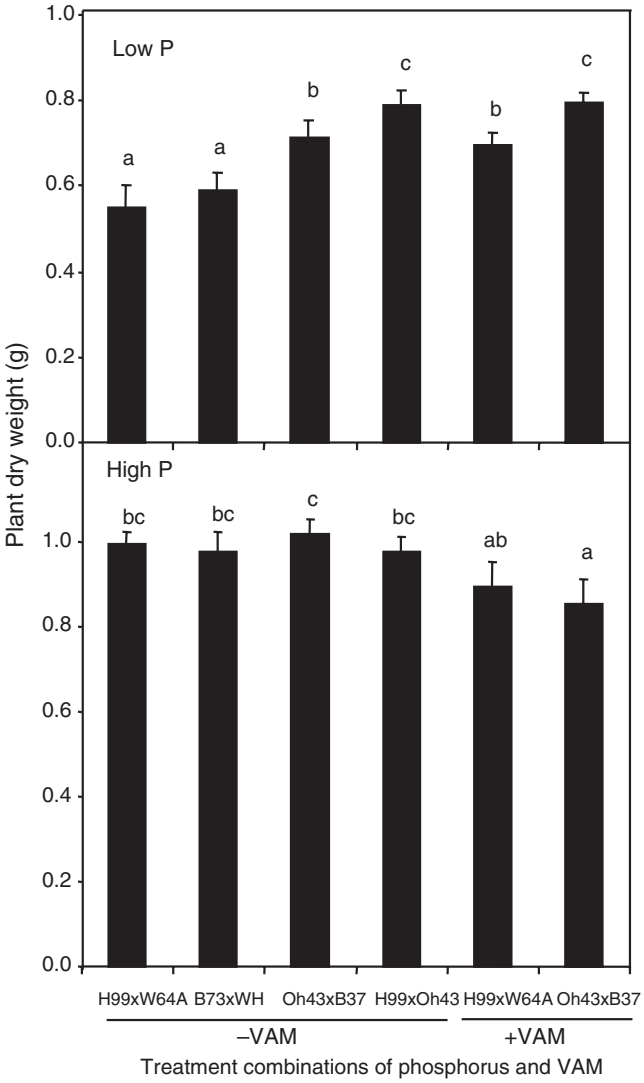

Fig. 3. Plant dry weight at 20 d after transplanting of four maize genotypes, as influenced by phosphorus availability in the growth media and mycorrhizal symbiosis. Data shown are means ± SE of the mean ($n = 4$). Means from low phosphorus or high phosphorus media with the same letter are not significantly different ($P \leq 0.05$).

AM symbiosis. Mycorrhizal inoculation reduced the biomass and relative growth rate of Oh43 × B37 at high phosphorus (Fig. 3; Table 1). Both shallow genotypes (Oh43 × B37 and H99 × Oh43) had significantly greater relative growth rates than the deep genotypes (H99 × W64A and B37 × WH) at low phosphorus availability (Table 1). Mycorrhizal inoculation reduced root : shoot ratios at high phosphorus for both genotypes and at low phosphorus for Oh43 × B37.

Phosphorus acquisition

At low phosphorus availability, shallow genotypes (Oh43 × B37 and H99 × Oh43) had greater shoot phosphorus content than the deep genotypes (H99 × W64A and B73 × WH) (Table 2). One of the shallow genotypes had the greatest specific phosphorus absorption rate at low phosphorus without mycorrhiza (Fig. 4). Mycorrhiza substantially increased specific phosphorus absorption rate at low phosphorus availability but decreased it at high phosphorus in the efficient genotype.

Shoot assimilation, shoot respiration, and root respiration

Low phosphorus availability decreased plant carbon budgets (Fig. 5). Genotypes with shallow root systems had more favourable net carbon balances than genotypes with deep root systems at low phosphorus availability (Fig. 5). The root respiration of deep genotype B73 × WH at low phosphorus consumed 47% of daily photosynthate, which was significantly higher than the two shallow genotypes, varying from 36–37% ($P<0.05$). However, another deep genotype H99 × W64A at low phosphorus consumed 39% of daily photosynthate, which was not significantly greater than the two shallow genotypes. There was no difference in root respiration costs on a per unit root dry weight basis at low phosphorus (data not shown). AM inoculation increased shoot assimilation and root respiration (Fig. 5). Mycorrhizal colonisation increased specific root respiration at low and high phosphorus (Fig. 6). Net daily carbon gain was closely correlated with absolute and relative growth rates (Fig. 7). Physiological efficiency of phosphorus acquisition, expressed as units of phosphorus acquired per unit of carbon respired by the root system, was greater in shallow-rooted genotypes than in deep-rooted genotypes at low phosphorus ($P<0.05$) (Fig. 8). Mycorrhizal inoculation decreased the physiological efficiency of phosphorus acquisition at low phosphorus ($P<0.05$) (Fig. 8).

Correlation and covariate analysis

Relative root length in the topsoil was positively correlated with shoot dry weight and net daily carbon gain at low phosphorus availability (Table 3). Mycorrhizal infection rate

Table 1. Biomass-based relative growth rate (RGR_{DW}, calculated according to Erickson 1976) and root shoot ratio (RSR) for maize genotypes measured at 20 d after planting, as influenced by phosphorus availability in the growth media and mycorrhizal symbiosis

VAM, vesicular-arbuscular mycorrhizae. Each value in the table is the mean of four replicates with SE. *F*-values are from general linear model ANOVA test (Minitab). Levels of significance are shown: *, $P<0.10$; **, $P<0.05$; ***, $P<0.01$; ns, not significant. For means from low phosphorus or high phosphorus media followed by same superscript letter are not significantly different ($P\leq0.05$)

Phosphorus availability	Genotype	Mycorrhizal symbiosis	RGR_{DW} ($mg\,g^{-1}\,d^{-1}$)	RSR ($g\,g^{-1}$)
Low	H99 × W64A	−VAM	59.4^{a}	0.60^{ab}
	B73 × WH	−VAM	59.4^{a}	0.68^{b}
	Oh43 × B37	−VAM	79.1^{b}	0.70^{b}
	H99 × Oh43	−VAM	89.2^{bc}	0.72^{b}
	H99 × W64A	+VAM	81.3^{b}	0.56^{a}
	Oh43 × B37	+VAM	96.8^{c}	0.55^{a}
High	H99 × W64A	−VAM	108^{d}	0.59^{c}
	B73 × WH	−VAM	97.7^{ab}	0.55^{bc}
	Oh43 × B37	−VAM	116^{b}	0.71^{d}
	H99 × Oh43	−VAM	112^{b}	0.52^{b}
	H99 × W64A	+VAM	93.1^{ab}	0.39^{a}
	Oh43 × B37	+VAM	90.7^{a}	0.38^{a}
F from ANOVA				
Block			0.75ns	3.15**
Genotype			2.64*	0.229ns
Phosphorus			6.18**	33.7***
VAM			0.00ns	40.4***
Genotype × phosphorus			1.07ns	2.88**
Phosphorus × VAM			9.57***	8.76***

Table 2. Shoot phosphorus content (SPC) and root phosphorus content (RPC) at 20 d after planting for maize genotypes measured, as influenced by phosphorus availability in the growth media and mycorrhizal symbiosis
VAM, vesicular-arbuscular mycorrhizae. Each value in the table is the mean of four replicates with SE. *F*-values are from general linear model ANOVA test (Minitab). Levels of significance are shown: *, $P<0.10$; **, $P<0.05$; ***, $P<0.01$; ns, not significant. For means from low phosphorus or high phosphorus media followed by same superscript letter are not significantly different ($P\leq0.05$)

Phosphorus availability	Genotype	Mycorrhizal symbiosis	Shoot phosphorus content (mg phosphorus plant^{-1})	Root phosphorus content (mg phosphorus plant^{-1})
Low	H99 × W64A	−VAM	0.520^{a}	0.166^{a}
	B73 × WH	−VAM	0.587^{a}	0.196^{ab}
	Oh43 × B37	−VAM	0.719^{b}	0.225^{ab}
	H99 × Oh43	−VAM	0.952^{b}	0.325^{b}
	H99 × W64A	+VAM	1.037^{b}	0.258^{ab}
	Oh43 × B37	+VAM	1.414^{c}	0.238^{ab}
High	H99 × W64A	−VAM	2.791^{a}	0.720^{b}
	B73 × WH	−VAM	2.725^{a}	0.702^{b}
	Oh43 × B37	−VAM	2.841^{a}	0.696^{b}
	H99 × Oh43	−VAM	3.245^{a}	0.390^{a}
	H99 × W64A	+VAM	2.896^{a}	0.681^{b}
	Oh43 × B37	+VAM	2.839^{a}	0.384^{a}
F from ANOVA				
Block			1.29ns	3.11*
Genotype			4.05**	1.41ns
Phosphorus			100.2***	29.8***
VAM			3.03*	1.4ns
Genotype × phosphorus			1.97ns	5.55***
Phosphorus × VAM			9.57***	4.81

was significantly associated with shoot phosphorus content at low phosphorus availability (Table 3). Root respiration was negatively associated with shoot dry weight and shoot phosphorus content (Table 3). Plant dry weight was significantly correlated with root dry weight (Fig. 9*a*), but root dry weight was not significantly associated with relative root length in the topsoil at $P<0.05$ (Fig. 9*b*). Covariate analysis showed root dry weight was non-significant for relative root length in the topsoil ($F=0.801$, $P=0.376$, data not shown).

Discussion

Our results show that genotype, phosphorus availability, and mycorrhizal symbiosis can modulate root shallowness in maize, and that root shallowness is important for plant adaptation to low phosphorus soils. Genotypes that exhibit a shallower root system were better adapted to low phosphorus environments in the field and under controlled conditions. Shallow genotypes had a greater specific phosphorus absorption rate, tissue phosphorus content, relative growth rate, net C assimilation rate and biomass accumulation after 20 d of growth than genotypes with deeper root systems. Our results support the following conclusions: (1) phosphorus availability modulates root shallowness in phosphorus inefficient genotypes; (2) mycorrhizal symbiosis modulates root shallowness; (3) modulation of root architecture by phosphorus availability and mycorrhizal symbiosis varies among plant genotypes; (4) root architecture and AM affect whole-plant carbon balance and (5) root shallowness is associated with phosphorus acquisition efficiency and adaptation to low phosphorus environments in maize.

Root shallowness is important for phosphorus acquisition efficiency in maize

The results from this study are consistent with previous reports of the importance of topsoil foraging for phosphorus acquisition in common bean (Lynch and Brown 2001; Miller *et al*. 2003). Phosphorus availability has been shown to regulate several aspects of root architecture in common bean, including axial extension, branching, basal root gravitropic setpoint angle, and relative distribution of basal root length and adventitious roots in the topsoil (Bonser *et al*. 1996; Liao *et al*. 2001; Lynch and Brown 2001; Ma *et al*. 2003; Miller *et al*. 2003). Since phosphorus availability is typically greater in the soil surface horizons in most natural and agricultural soils, topsoil foraging has been proposed as an important means for plants to improve phosphorus acquisition efficiency (Lynch and Brown 2001). In other words, strategies that result in a shallower root system and / or the ability of a root system to plastically respond to a low phosphorus environment by becoming shallower,

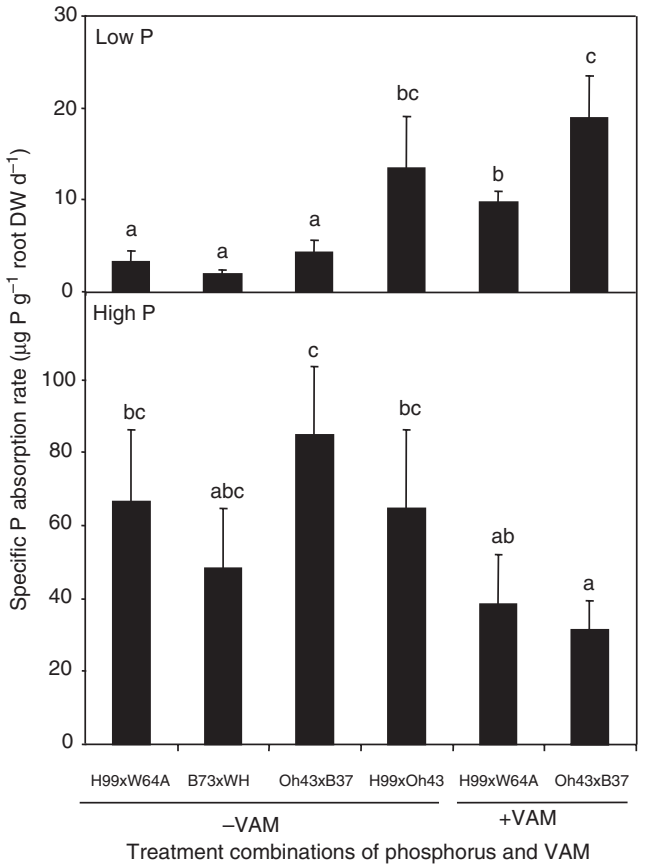

Fig. 4. Specific phosphorus absorption rate from day 10 to day 20 after transplanting of four maize genotypes, as influenced by phosphorus availability in the growth media and mycorrhizal symbiosis. Data shown are means ± SE of the mean ($n = 4$). For means from low phosphorus or high phosphorus media with the same letter are not significantly different ($P \leq 0.05$, ANOVA *post hoc* test).

should have improved phosphorus acquisition, relative to deep-rooted architecture strategies.

In this study, we confirm that root shallowness is indeed important for plant adaptation to low phosphorus in maize. Root shallowness was significantly correlated with plant growth in the field among contrasting genotypes (Fig. 1). Maize genotypes that were shallower and phosphorus efficient in the field similarly had a greater biomass accumulation (Figs 2, 3) and greater root and shoot tissue phosphorus content (Table 2) when grown under low phosphorus availability in sand-culture in the greenhouse for 3 weeks. In the greenhouse study, the phosphorus efficient genotypes had a greater proportion of root length localised in the surface layer (Fig. 2), as was also observed in the field (Fig. 1). The fact that root shallowness was important for phosphorus acquisition in both field and greenhouse environments means that the greenhouse conditions were realistic simulations of the field while permitting the exclusion of potentially confounding variables, and also that this relationship is robust across environments. Our sand culture system buffered phosphorus supply in a manner that mimics the phosphorus availability and physical impedance of natural soil (Lynch *et al*. 1990; Zhu and Lynch 2004), which appears to be an appropriate approach to screen promising phosphorus efficient genotypes in maize. The phosphorus-efficient genotypes also produced more total root length under low phosphorus conditions than the phosphorus-inefficient genotypes, although there was no difference among genotypes in total root length under high phosphorus conditions (Fig. 2). The greater root length of phosphorus-efficient genotypes under low phosphorus conditions is explained by their greater overall biomass and the consistent allometric relationship of plant biomass with root biomass (Fig. 9). The greater accumulation of biomass and phosphorus in phosphorus-efficient genotypes was likely due to the fact that these plants allocated a greater fraction of that total root length to the surface horizon under low phosphorus conditions. Previous studies have similarly found that root shallowness in low phosphorus conditions was positively correlated with tissue phosphorus content and plant biomass accumulation in common bean (Bonser *et al.* 1996; Liao *et al*. 2001, 2004). In addition, results from dynamic geometric modelling of bean root systems suggest that shallower root systems should have greater phosphorus acquisition per unit of carbon allocation, compared with deeper root systems, particularly for older plants, because of enhanced topsoil foraging as well as reduced inter-root competition (Ge *et al*. 2000). This prediction was supported by our present results, which show that shallow genotypes had higher physiological efficiency of phosphorus acquisition, in units of phosphorus acquired per unit of C respired by roots, compared with the deep genotypes at low phosphorus (Fig. 8).

Although shoot growth was highly correlated with root growth, it was not correlated with root shallowness (Fig. 9). Covariate analysis showed that there was no significant relationship found between total root dry weight and relative root length fraction in the shallow layer (F=0.801, P=0.376). In other words, relative root shallowness was not driven by the size of the root system or the size of the plant. Hence, spatial localisation of roots in surface horizons is an important mechanism for improving phosphorus acquisition efficiency in low phosphorus environments.

Plasticity of root shallowness in response to phosphorus availability

Low phosphorus availability had no effect on root shallowness in phosphorus-efficient genotypes, but reduced root shallowness in the phosphorus-inefficient genotypes (Fig. 2*a*). The lack of effect of phosphorus availability in shallow rooted genotypes may indicate that

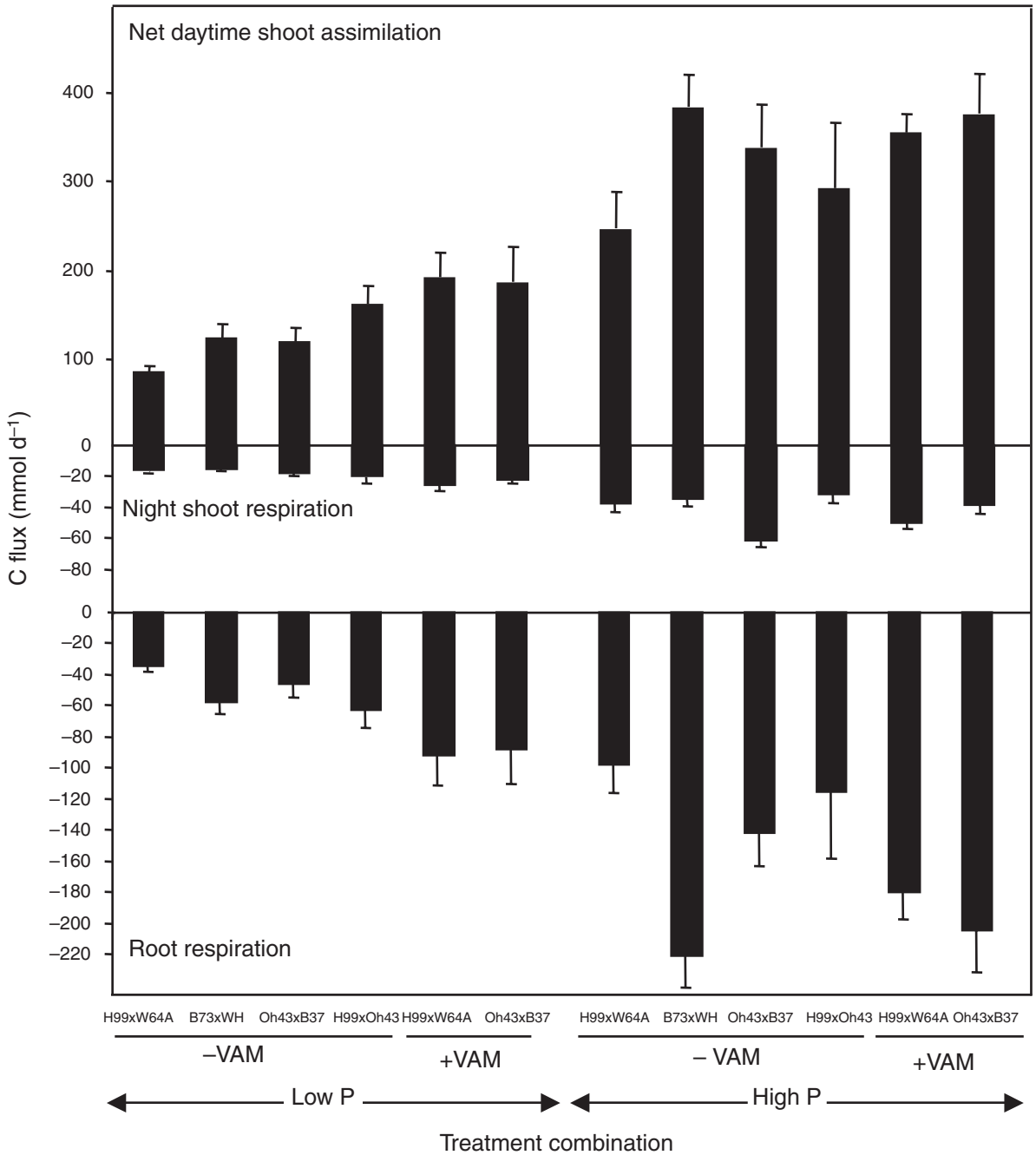

Fig. 5. Shoot assimilation rate, shoot respiration rate, and root respiration rate of maize genotypes at 20 days, as influenced by phosphorus availability in the growth media and mycorrhizal symbiosis. Data shown are means ± standard error of the mean ($n = 4$).

there is an architectural limit to root shallowness, and that the phosphorus-efficient, shallow-rooted genotypes had already reached that limit. Another possibility is that architectural plasticity in response to phosphorus availability is a genetically controlled trait, and that the phosphorus-efficient genotypes employed here were coincidentally non-plastic.

Mycorrhizal symbiosis affects root shallowness and phosphorus acquisition efficiency

We found that mycorrhizal symbiosis affected root architecture and phosphorus acquisition efficiency of maize, but the effect of AM was dependent upon the genotype and phosphorus treatment. High phosphorus availability reduced mycorrhizal colonisation, perhaps because the carbon costs

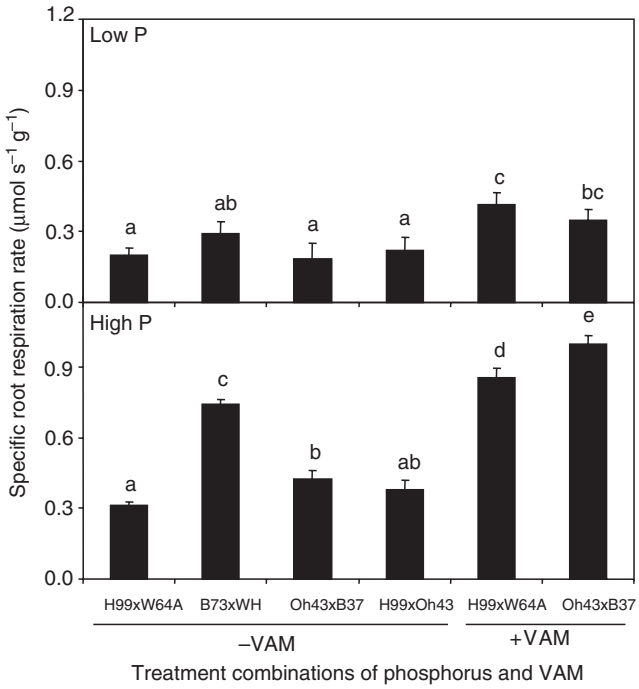

Fig. 6. Specific root respiration per unit of root dry weight of four maize genotypes, as influenced by phosphorus availability in the growth media and mycorrhizal symbiosis. Data shown are means ± SE of the mean ($n = 4$). Means from low phosphorus or high phosphorus media with same letter are not significantly different ($P \leq 0.05$).

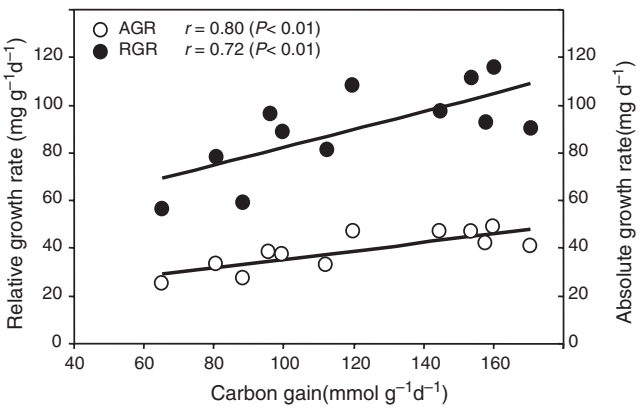

Fig. 7. Correlation of net daily carbon gain with absolute growth rate and relative growth rate in four maize genotypes at varying phosphorus availability and mycorrhizal status.

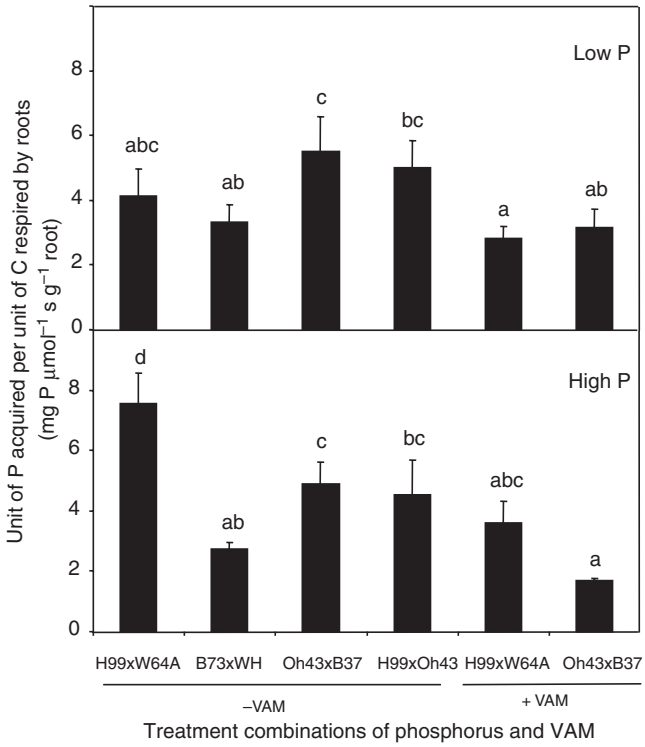

Fig. 8. Mean of units of phosphorus acquired per unit of C respired by roots at day 20 after transplanting of four maize genotypes, as influenced by phosphorus availability in the growth media and mycorrhizal symbiosis. Data shown are means ± SE of the mean ($n = 4$). For means from low phosphorus or high phosphorus media with the same letter are not significantly different ($P \leq 0.05$, ANOVA *post hoc* test).

associated with maintaining the fungal tissue outweighed the benefit of obtaining additional phosphorus. The lack of benefit from mycorrhiza was evident in the decrease in specific phosphorus absorption rate (Fig. 4), decrease in relative growth rate (Table 1), and consequently the decrease in biomass of +AM plants, relative to −AM grown at high phosphorus. In addition, under high phosphorus, the root architecture was not greatly affected by AM, as there was no significant change in the total root length or the relative fraction of roots in the surface layer for either genotype, when roots were associated with or without AM.

Mycorrhizal symbiosis did alter root architecture and increased phosphorus acquisition of plants under low phosphorus conditions. Mycorrhizal symbiosis increased the specific phosphorus absorption rate (Fig. 4), which resulted in an almost doubling of the shoot phosphorus content (Table 2) and greater plant biomass (Fig. 3) for both maize genotypes inoculated with AM, when grown under low phosphorus conditions. Nielsen *et al.* (1998) also showed enhanced specific phosphorus absorption rates due to mycorrhizal symbiosis in common bean under low phosphorus availability. Mycorrhiza, however, did not have the same effect on root architecture for the two maize genotypes under low phosphorus. The shallow, phosphorus-efficient genotype responded to low phosphorus by increasing the total and relative fraction of roots in the surface layer, while with AM, this response did not occur. When inoculated with AM, the deep, phosphorus-inefficient genotype (H99 × Oh43) behaved more like the shallow,

Table 3. Correlation (Pearson linear correlation coefficient, *r*, df = 22) among total root length (TRL), relative root length in the topsoil (RRL), shoot dry weight (SDW), shoot phosphorus concentration (SPCO), root phosphorus concentration (RPCO), AM infection rate (AMIR), intact root respiration (IRR), and net C gain (NCG) at 20 d after planting for maize genotypes at low phosphorus media

Levels of significance and indicated: *, $P<0.05$; **, $P<0.01$

	TRL	RRL	SDW	SPCO	RPCO	AMIR	IRR
RRL	0.727**	–	–	–	–	–	–
SDW	0.556**	0.624**	–	–	–	–	–
SPCO	0.484	0.45	0.835**	–	–	–	–
RPCO	0.042	0.133	0.222	0.134	–	–	–
AMIR	−0.103	0.052	0.465	0.614**	0.125	–	–
IRR	−0.321	−0.268	−0.715**	−0.818**	0.073	−0.503	–
NCG	0.402*	0.589**	0.513**	0.651**	−0.156	0.329	−0.856**

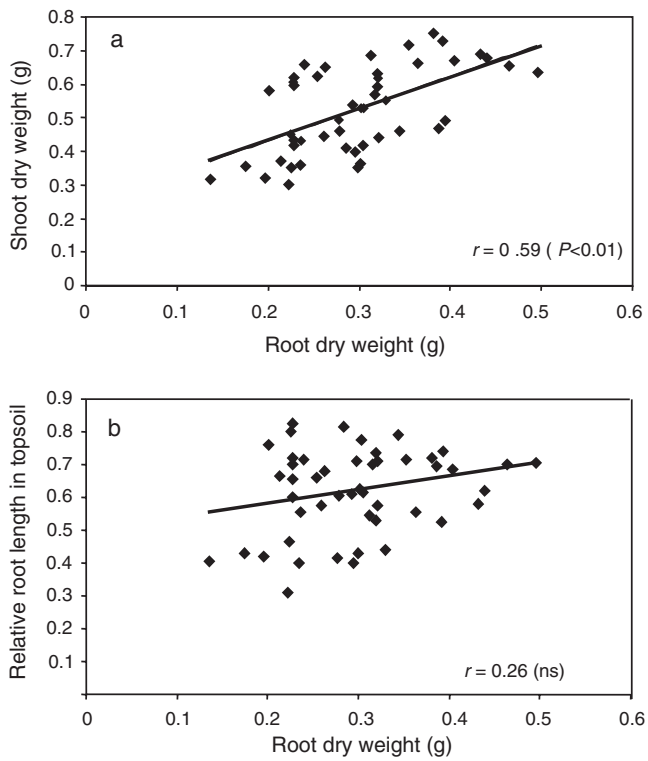

Fig. 9. Correlation of root dry weight and shoot dry weight (*a*), and root dry weight and relative root length in topsoil (*b*) for four maize genotypes at 20 d after transplanting. Covariate analysis showed root dry weight was non-significant for relative root length of upper layer (F=0.801, P=0.376).

phosphorus-efficient genotypes under low phosphorus by increase the relative fraction of roots in the topsoil. The ultimate performance ranking of the two genotypes was not affected by AM. Yan *et al*. (1995) similarly found in common bean that while mycorrhizal infection affected the overall phosphorus acquisition between genotypes, the ranking of genotypes in terms of biomass production under low phosphorus conditions did not change. In the case of maize, we also speculate that inherent root traits, rather than symbiotic efficiency govern the adaptation of a genotype to low phosphorus environments.

Phosphorus availability and mycorrhizal colonisation affect whole-plant carbon balance

Both phosphorus availability and mycorrhizal colonisation significantly affected whole-plant carbon balance. When phosphorus availability was low, total C assimilation decreased for all four genotypes, though the decrease was not as dramatic for phosphorus-efficient genotypes (Fig. 5). Respiration costs of roots and shoots of phosphorus-efficient genotypes were significantly lower under low phosphorus conditions compared with the inefficient genotypes. We also found the percentages of C used in whole-root respiration in all genotypes were maintained under low and high phosphorus availability. Nielsen *et al*. (1998, 2001) reported that low phosphorus availability significantly increased the percentages of C used in whole-root respiration in both phosphorus-efficient and phosphorus-inefficient genotypes at 28 and 35 d after planting (DAP) in common bean, although this was not evident at 14 and 21 DAP, which is consistent with our present results with young maize seedlings. In addition, our results also show that shallow, phosphorus-efficient genotypes have a lower relative fraction of C going to respiratory costs under low phosphorus conditions. Physiologically and metabolically this would be expected, as there is likely a greater net return of phosphorus acquisition for every unit of C expended for growth and maintenance of the root system, when roots are distributed in the region where phosphorus is localised in the environment.

Mycorrhizal symbiosis also affected whole-plant carbon balance. For both genotypes, mycorrhizal inoculation resulted in increased C assimilation rates at low phosphorus treatments, but not at high phosphorus (Fig. 5). Root and shoot respiration rates also increased for +AM plants, with the exception of shoot respiration of Oh43 × B37, which

was actually lower for −AM plants under high phosphorus (Fig. 5). For high phosphorus treatments, the high respiratory costs of AM outweighed the benefits of increased phosphorus acquisition. For both genotypes inoculated with AM, SPAR (Fig. 4) and the final plant dry weight (Fig. 4) decreased under high phosphorus for +AM plants relative to −AM plants. The opposite is true for low phosphorus, +AM treatments, where the benefits of the mycorrhizal association are ultimately greater for the plant than the costs. Nielsen *et al*. (1998) showed a comparable increase in the respiration of the whole root system when plants were inoculated with AM. Though root and shoot respiration costs also increase for +AM plants under low phosphorus conditions, biomass accumulation (Fig. 3) and SPAR (Fig. 4) are greater for plants associated with AM than without AM for both genotypes. Root strategies that enable a plant to maximise net C gain are likely associated with adaptation to low phosphorus environments. Both root shallowness and association with mycorrhizas are important mechanisms for improving the net C gain of a plant because they facilitate more efficient phosphorus acquisition when phosphorus is limiting.

Genetic variation in root shallowness may represent adaptation to various selection regimes. The primary abiotic constraints encountered in maize environments include low phosphorus, wind and drought (Ribaut *et al*. 1996; Goodman and Ennos 1997; Kaeppler *et al*. 2000). Rooting depth has important implications for ecosystem hydrological balance and for wind resistance (Bardet *et al*. 1996; Canadell *et al*. 1996). Economic optimisation modelling indicates that rooting depth is subject to tradeoffs for water and phosphorus acquisition (Ho *et al*. 2004). Bean genotypes with shallow basal roots had superior phosphorus acquisition, whereas those with deep roots had superior drought tolerance in field and greenhouse conditions (Ho 2005). The influence of root architecture on interplant competition is an important aspect of stand performance. Both geometric modelling and field studies indicate that shallow-rooted genotypes of bean are more competitive for phosphorus than deep-rooted genotypes in polygenetic stands (Rubio *et al*. 2003). The importance of root architecture for interplant competition under drought stress remain to be determined but are probably at least as significant as effects under phosphorus stress, since competition for water extends over longer distances than competition for phosphorus. We propose that genotypic variation for rooting depth in maize represents adaptation to distinct abiotic stress regimes. Shallow-rooted genotypes may be suited to moist but infertile environments, whereas deep-rooted genotypes may be better adapted to drought. In maize, the effect of the depth of the primary root system on wind resistance may be secondary to the effects of adventitious roots.

We observed substantial genetic variation for root shallowness among the four genotypes in maize under low phosphorus conditions. Our results also demonstrate the physiological utility of root shallowness under low phosphorus conditions, and that mycorrhizal symbiosis does not negate this utility. Because the parents of these genotypes are common in the genetic background of current commercial hybrids, this information might be readily applicable to maize breeding and research programs. We suggest that variation in root shallowness may be harnessed to improve the phosphorus efficiency of maize in low phosphorus habitats or low input agricultural systems.

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