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Growth, nutrition, and soil respiration of a mycorrhiza-defective tomato mutant and its mycorrhizal wild-type progenitor

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Abstract. The effects of colonisation of roots by arbuscular mycorrhizal fungi (AMF) on soil respiration, plant growth, nutrition, and soil microbial communities were assessed using a mycorrhiza-defective tomato (*Solanum lycopersicum* L.) mutant and its mycorrhizal wild-type progenitor. Plants were grown in rhizocosms in an automated respiration monitoring system over the course of the experiment (79 days). Soil respiration was similar in the two tomato genotypes, and between P treatments with plants. Mycorrhizal colonisation increased P and Zn content and decreased root biomass, but did not affect aboveground plant biomass. Soil microbial biomass C and soil microbial communities based on phospholipid fatty acid (PLFA) analysis were similar across all treatments, suggesting that the two genotypes differed little in their effect on soil activity. Although approximately similar amounts of C may have been expended belowground in both genotypes, they may have differed in the relative C allocation to root construction *v.* respiration. Further, net soil respiration did not differ between the two tomato genotypes, but root dry weight was lower in mycorrhizal roots, and respiration of mycorrhizal roots per unit dry weight was higher than nonmycorrhizal roots. This indicates that the AM contribution to soil respiration may indeed be significant, and nutrient uptake per unit C expenditure belowground in this experiment appeared to be higher in mycorrhizal plants.

Additional keywords: mycorrhiza mutant, mycorrhizas, PLFA, respiration, roots, root respiration, *Solanum lycopersicum*.

Introduction

Soils play an important but poorly understood role in the global carbon budget. Plant-derived C enters the soil as plant litter and root exudates (Coleman *et al*. 1976; Butler *et al*. 2003), and is released as CO_2 by the respiration of roots and soil microorganisms. As much as 50% of soil CO_2 efflux is root-derived (Rochette and Angers 1999; Kuzyakov and Domanski 2002), with contributions coming directly from root respiration and indirectly from heterotrophic respiration of C originating as root exudates, exfoliates (sloughed cells and root hairs), and root debris (Ingham *et al*. 1985; Cheng 1996; Luo *et al*. 1996; Kuzyakov and Domanski 2002; Langley *et al*. 2005). Roots may also alter soil respiration via inorganic carbon fixation in the root zone (Cramer *et al*. 1993).

The arbuscular mycorrhizal fungi (AMF) that colonise the roots of most terrestrial plant species can influence soil carbon processes in a variety of ways and at a range of scales from the individual plant to the ecosystem (Rillig 2004). Primary among these influences is improved plant nutrition (Marschner and Dell 1994; Smith and Read 1997) which can lead to increased growth and soil C inputs. AMF can also stabilise soil aggregates (Rillig 2004), and this may slow soil C turnover and increase long-term C storage. AMF may also influence rates of soil CO_2 efflux from soils, both directly via their own metabolism (Pang and Paul 1980; Peng *et al*. 1993; Valentine and Kleinert 2007) and indirectly via their effects on root growth and metabolism as well as by heterotrophic consumption of AMF-derived C (Langley *et al*. 2005), for example, by collembolans and nematodes (Gange 2000; Bakhtiar *et al*. 2001).

Colonisation of roots by AMF can alter plant C allocation (Jakobsen and Rosendahl 1990) (depending on developmental stage Mortimer *et al*. 2005), and stimulate plant photosynthetic rates (Smith and Read 1997). Allocation of C to AMF may vary with stage of plant development (Mortimer *et al*. 2005). Although some studies have shown that colonisation of roots by AMF can increase belowground respiration (Pang and Paul 1980; Peng *et al*. 1993), others have suggested otherwise (Silsbury *et al*. 1983). Reasons for such differences remain unknown, although

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size differences between mycorrhizal and non-mycorrhizal plants are likely an important factor in determining total belowground respiration (Langley *et al*. 2005). Methodological artefacts may also contribute to the observed variation in the effects of AMF on belowground respiration. Sterilisation or fumigation of soils is commonly used to establish controls in mycorrhizal studies, yet can cause elimination of non-target members of the soil biota (see Cavagnaro *et al*. 2006; for discussion). This may lead to underestimation of the heterotrophic component of soil respiration, and hence, overestimation of the mycorrhizal contribution to soil respiration, with an uncertain influence on total belowground respiration. To avoid such potential problems, a mycorrhiza-defective tomato mutant (*rmc*) and its mycorrhrizal wild-type progenitor (76R MYC+) (Barker *et al*. 1998) have been used in both glasshouse and field settings to study the effects of AMF on plant growth and nutrition (Gao 2002; Poulsen *et al*. 2005; Cavagnaro *et al*. 2006), plant ecology (Cavagnaro *et al*. 2004), and responses to elevated atmospheric CO_2 concentrations (Cavagnaro *et al*. 2007*b*). The growth of the two genotypes has been found to be very similar under a range of circumstances (Cavagnaro *et al*. 2004, 2006; Poulsen *et al*. 2005), including non-mycorrhizal conditions, suggesting that the mutation affecting colonisation of *rmc* by AMF has no pleiotropic effects on other plant processes (Cavagnaro *et al*. 2004).

Recently, Langley *et al*. (2005) used an automated respiration monitoring system which measures soil respiration with a high degree of temporal resolution, with and without plants and AMF, over the entire course of a plant's growth cycle. This unique experimental system allows for averaging of diurnal effects on soil respiration, and assessing the impacts of both abiotic and biotic factors on soil respiration. Using this system, we compared plant growth and nutrition, soil microbial biomass and community profiles, and belowground respiration in mesocosms, of *rmc* and 76R MYC+ tomato genotypes to avoid disturbance of the wider soil biota (Cavagnaro *et al*. 2006). In order to influence the potential nutritional benefits of mycorrhizal status, we crossed two levels of phosphorus availability with the two tomato genotypes. Specifically we addressed the following questions:

(1) does respiration of mycorrhizal and non-mycorrhizal root systems of otherwise similar plants differ; and
(2) does the presence of roots, colonised by AMF or not, alter soil microbial biomass C (MBC) or microbial community composition (PLFA profiles)?

Materials and methods

Soil collection, mixing and nutrient addition

The sandy loam soil used in this study was from the Epikom series (loamy, mixed, superactive, mesic Lithic Haplocambids) collected from a desert grassland 40 km north of Flagstaff, Arizona, and similar to that used in earlier work (Langley *et al*. 2005). The soil was chosen to facilitate root removal due to its sandy texture, and because its low C content was expected to decrease background heterotrophic respiration. The soil was mixed in a 1 : 2 ratio with sand. Physiochemical properties of the final soil : sand mix, including soil particle size distribution, pH, exchangeable Na, exchangeable K, exchangeable Mg, exchangeable Ca, total N, total C and DPTA extractable Zn, Mn, Cu and Fe were determined (Table 1), by the DANR Analytical Laboratory, University of California Davis (http://danranlab.ucanr.org, verified 17 March 2008). K_2HPO_4 was mixed with the soil to establish high (7.5 μg P added/g soil) and low (1.5 μg P added/g soil) P treatments. One hundred millilitres of the appropriate P solution was added in a drop-wise manner to 90 kg of soil, spread out on a plastic sheet. The solution was then 'rubbed' into the soil by hand, and the soil thoroughly mixed for several minutes. Final plant available (Olsen) P concentrations in 'low P' and 'high P' treatments were 104 ± 2 and 113 ± 2 μg P/g soil (mean ± s.e.), respectively, as determined by the same laboratory. Although initial soil P concentrations were higher than anticipated, the difference between the two P addition treatments were significant, as were plant responses to them (see Results). This soil type, and other related desert soils, can be high in total P, although available P is typically lower than found here (Krämer and Green 1999). The soil was packed into plastic free-draining pots. Each pot contained 4.5 kg of soil : sand mix, on a dry weight basis. Eighteen pots of each P treatment were established.

Plants

Seeds of the mycorrhiza-defective tomato mutant (*rmc*), and its mycorrhizal wild-type progenitor *S. lycopersicum* cv. 76R (76R MYC+) (Barker *et al*. 1998), were surface-sterilised and pre-germinated following Cavagnaro *et al*. (2006). Seeds germinated within ~5 days, after which they were planted in peat moss in seedling trays and grown in a glasshouse for 26 days, followed by hardening in a lath house for 21 days. These seedlings were then used in the main experiment.

Experimental design

An experiment was established as a randomised complete block design on 6 July 2004. There were two soil P amendment treatments (high or low P, see above) and three plant treatments: 76R MYC+, *rmc*, or plant free soil controls (soil blanks hereafter), applied in combination, giving a total of 36 pots filled with soil. An additional six replicates were

Table 1. Soil characteristics of the soil : sand mix

Soil physical and chemical properties	Mean ± s.e. ($n = 4$)
Sand (%)	80.9 (0.25)
Silt (%)	10.4 (0.45)
Clay (%)	8.8 (0.5)
pH	6.7 (0.0)
Total N (%)	0.1 (0.01)
Total C (%)	0.3 (0.05)
C : N ratio	5.7 (0.2)
Exch. K (meq/100 g)	0.6 (0.02)
Exch. Na (meq/100 g)	0.1 (0.01)
Exch. Ca (meq/100 g)	9.5 (0.32)
Exch. Mg (meq/100 g)	2.6 (0.12)
Extractable Zn DPTA (ppm)	0.3 (0.03)
Extractable Mn DPTA (ppm)	21.3 (3.9)
Extractable Cu DPTA (ppm)	1.0 (0.06)
Extractable Fe DPTA (ppm)	40.9 (7.7)
P (Olsen), plant available, low P treatment (ppm)	104.2 (2.2)
P (Olsen), plant available, high P treatment (ppm)	113.4 (2.1)

included in the experiment which consisted of pots without plants or soil (chamber blanks hereafter). Following planting, pots were placed in sealed belowground rhizocosms to monitor soil respiration.

Automated respiration monitoring system

Soil respiration was monitored over the course of the experiment using the automated respiration monitoring system at Northern Arizona University. A detailed description of the glasshouse layout and respiration chambers (rhizocosms) is given by Langley *et al*. (2005). Briefly, immediately following planting the pots were placed in rhizocosms, constructed of air-tight, white PVC cylinders (50 cm height × 13 cm diameter). The top of each chamber was sealed around the plant stem with a split rubber stopper with a centre hole and gas-impermeable putty (Quibitac, Qubit Systems, Kingston, ON, USA). Six pots from each of the six experimental treatments (see above) and six completely empty pots (chamber blanks) were each assigned to 1 of 42 monitored rhizocosms. A program written and run in LabView (National Instruments, Austin, TX, USA) controlled a Hewlett-Packard data acquisition system (HP3495) that managed air handling and signal acquisition. CO_2 concentration of air drawn from above the glasshouse and delivered to the inlet port on each rhizocosm (reference [CO_2]) was measured using an infrared gas analyser (IRGA, Model 6262, Li-Cor, Lincoln, NE, USA). The concentration of CO_2 exiting each microcosm was measured by the IRGA ~every 2 h (sample [CO_2]). A mass flow meter (Model 820, Sierra Instruments, Monterey, CA, USA) measured the flow rate through each container (~400 mL min^{-1}). CO_2 flux (soil respiration) from rhizocosms was calculated following Eqn 1:

$$\text{soil respiration} = \text{flow rate} \times (\text{sample } [CO_2] - \text{reference } [CO_2]). \quad (1)$$

Pots containing either soil or soil and plants were weighed and watered regularly to maintain the weight corresponding to 11% gravimetric moisture. Once a week, plants were also supplied with 30 mL of a modified Long Ashton solution minus P (Cavagnaro *et al*. 2001).

Plant sampling

Plants were destructively harvested 79 days after planting on 22 September 2004. The soil chambers were disassembled and the experimental pots removed. Plant shoots were immediately cut at the soil surface and fresh weights determined. The shoots were separated into stems and leaves, dried at 60°C, weighed, and ground for nutrient analysis. Shoot and fruit B, Ca, Fe, K, Mg, Mn, Na, P and Zn contents were determined on plant material that was microwave-digested with nitric acid (Sah and Miller 1992) and analysed by ICP-AES (Thermo Jarrell Ash Corp., Franklin, MA, USA). Stable isotope ratios, and concentrations, of C and N were measured by mass spectrometry at the University of California Davis Stable Isotope Facility (http://stableisotopefacility.ucdavis.edu/, verified 17 March 2008) as described by Cavagnaro *et al*. (2006).

Soil was removed by cutting the pot away from the soil mass, which retained its form. Soils from 0 to 8 cm and 8–30 cm depth were mixed separately by hand for 30 s, subsamples were taken, and soil analyses performed as outlined below. Roots were extracted from each zone using a combination of dry picking with forceps and wet sieving, and root length determined using a root length scanner (Comair, Melbourne, Victoria, Australia) and the grid line intersect method (Tennant 1975). A subsample of roots was dried at 60°C and dry weights determined. Mycorrhizal colonisation of roots was determined using a modified grid-line intersect technique as described previously (Cavagnaro *et al*. 2006).

Soil sampling

Soil from the 0–8 cm soil zone was subsampled as follows. Samples for PLFA community analysis were taken first; aggregates (5–10 mm in diameter) were collected and transferred to tubes for storage at –20°C. Phospholipid fatty acid (PLFA) analysis was performed using a modified chloroform-methanol extraction (Bligh and Dyer 1959; Bossio and Scow 1998) with transesterification of the polar lipid fraction containing the phospholipids (Guckert *et al*. 1986), as described by Cavagnaro *et al*. (2006). Only the low P samples were used for PLFA analysis because these treatments were expected to show the greatest mycorrhizal dependence. Duplicate subsamples from each soil sample (25 g moist soil) were analysed for microbial biomass C (MBC) by the fumigation extraction method (Vance *et al*. 1987). Triplicate soil samples (30 g moist soil) were extracted with 2 M KCl, and inorganic N content was determined colourimetrically using a modification of Miranda *et al*. (2001) for NO_3^- (plus NO_2^-) and Forster (1995) for NH_4^+. A 50-g subsample of soil from both soil zones was taken for determination of gravimetric moisture content after drying at 105°C for 48 h.

Calculations and statistical analysis

Plant and soils data were analysed using the General Linear Model (see exceptions below) in SAS (version 8.02, SAS Institute, Cary, NC, USA). Where significant effects were observed, pair-wise comparisons were made using Tukey's test (Zar 1999). Mycorrhizal colonisation data were arcsine transformed (Zar 1999) before analysis, and are presented as such.

Mean hourly respiration data were used to calculate CO_2 respired each day, and thence, cumulative respiration (CR) on day Y was calculated following Eqn 2.

$$CR_Y = \Sigma(X_{DAY\,1} + X_{DAY\,2} \ldots + X_{DAY\,Y}), \quad (2)$$

where X = the total CO_2 respired on a given day.

Soil respiration data were analysed as repeated-measures using the ANOVA procedure in SAS. All respiration data are presented starting from day 16 as data before this were highly variable due to the small size of the plants and technical problems with the gas handling system.

Results

Soil respiration

Cumulative respiration increased linearly over the course of the experiment (Fig. 1). Total respiration of rhizocosms in soil without plants was ~30% of that in rhizocosms containing

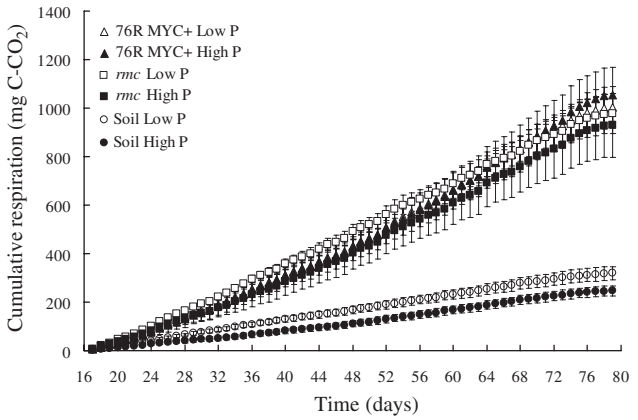

Fig. 1. Cumulative CO_2 respired over the course of the experiment (mg C-CO_2) in rhizocosms. Treatments were: soil only/low P, soil only/high P, *rmc*/low P, *rmc*/high P, 76R MYC+/low P, and 76R MYC+/high P. Low P and High P refer to P1.5 and P7.5 treatments, respectively, i.e. 1.5 and 7.5 μg P added/g soil. Values are mean ± s.e., $n = 6$.

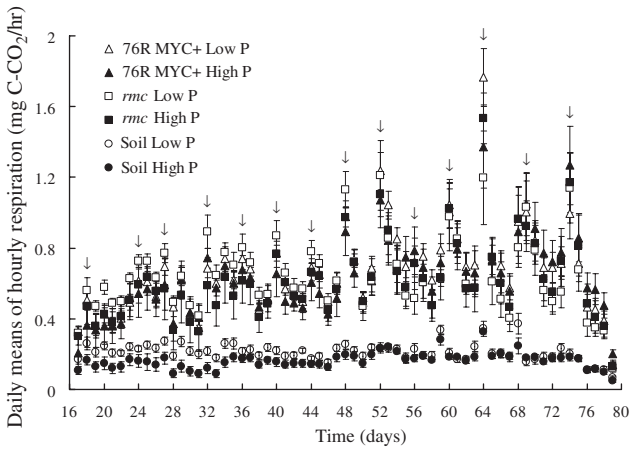

Fig. 2. Daily mean of hourly respiration (mg C-CO_2/h) from rhizocosms. Treatments were: soil only/low P, soil only/high P, *rmc*/low P, *rmc*/high P, 76R MYC+/low P, and 76R MYC+/high P. Low P and High P refer to P1.5 and P7.5 treatments, respectively, i.e. 1.5 and 7.5 μg P added/g soil. Arrows indicate time of watering. Values are mean ± s.e., $n = 6$.

plants. In rhizocosms containing soil only, cumulative respiration increased significantly with time ($P < 0.0001$) and was lower in the high P addition treatment ($P = 0.03$). In these rhizocosms, daily means of hourly respiration rates showed a significant interaction between time and P addition treatment ($P = 0.01$), explained by a gradual decrease in the difference between the two P levels with time (Fig. 2).

In rhizocosms containing plants, cumulative respiration increased significantly with time ($P < 0.0001$), but there were no differences between genotypes or P addition treatments (Fig. 1). This was also true for daily means of hourly respiration (Fig. 2). Spikes in soil CO_2 emissions closely followed watering of pots (arrowed in Fig. 2). The amount of CO_2 respired per hour (averaged over the last 5 days of the experiment; see Fig. 2) per unit root dry weight at harvest (see below; Fig. 3*c*) was significantly ($P = 0.0138$) higher in the 76R MYC+ plants than *rmc* plants, irrespective of P addition treatment.

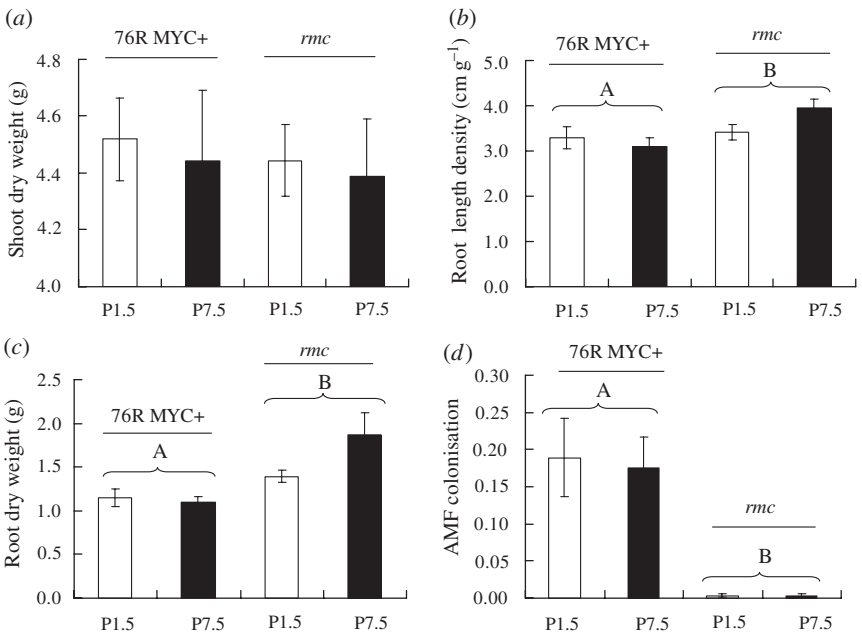

Fig. 3. (*a*) Shoot dry weight (g), (*b*) root length density (cm g^{-1} dry soil), (*c*) root dry weight (g), and (*d*) mycorrhizal colonisation at harvest (percent arcsine transformed), of 76R MYC+ and *rmc* tomato plants grown in the low (P1.5) and high (P7.5) P addition treatments, respectively, i.e. 1.5 and 7.5 μg P added/g soil. Values are mean ± s.e., $n = 6$. Different letters indicate significant differences at $P < 0.05$ for genotype main effect.

Table 2. Soil microbial biomass and mineral N pools
Microbial biomass C (MBC), NH_4^+-N concentration (μg/g) and NO_3^--N concentration (μg/g soil) in the surface 0–15 cm, $n = 6$

	MYC[A] P1.5		MYC P7.5		*rmc* P1.5		*rmc* P7.5		Soil P1.5		Soil P7.5	
	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.	Mean	s.e.
MBC (μg/g)	191.5	24.2	197.1	18.9	159.1	17.4	173.7	8.6	219.4	21.5	198.7	24.9
NH_4^+-N (μg/g)	0.6	0.1	0.7	0.1	0.7	0.1	0.7	0.1	0.4	0.1	0.6	0.2
NO_3^--N (μg/g)	0.02	0.01	0	0	0.02	0.02	0.02	0.02	6.3	0.6	5.4	0.7

[A]MYC = 76R MYC+.

Soil microbial biomass, PLFA and mineral N pools

Microbial biomass C was not influenced by the P addition (low or high) or plant (76R MYC+, *rmc*, soil only) treatments, alone or in combination (Tables 2, 3). The total PLFA was not different between treatments ($P < 0.6$), with a mean (±s.e.) value of 2045 (±317) ng/g soil. Based on multivariate statistical analysis of PLFA profiles, soil microbial communities did not differ consistently between any of the low P experimental treatments (data not shown). This analysis included the 29 PLFA that were present in more than 10% of samples.

Soil NO_3^- concentrations (Tables 2, 3) were higher in the soil blanks (≥5.8 μg N/g soil) than in the treatments with plants (≤0.02 μg N/g soil). There were no differences in soil NH_4^+ concentrations (Tables 2, 3), which were ≤0.7 μg N/g soil in response to any of the experimental treatments.

Plant growth, nutrient uptake and mycorrhizal colonisation

Shoot dry weights of 76R MYC+ and *rmc* plants did not differ at harvest (Fig. 3*a*), whereas root length density (Fig. 3*b*) and root dry weights (Fig. 3*c*) were higher for *rmc* plants. Total biomass (shoots + roots) did not differ between genotypes or P addition treatments (data not shown). AMF colonised both the epidermal and cortical cell layers of the 76R MYC+ roots (~20% root length colonised), whereas colonisation of *rmc* was restricted to the root epidermis and remained low (less than 1%) (Fig. 3*d*).

Shoot concentrations of Zn (Fig. 4*a*) and P (Fig. 4*b*) were 58 and 20% higher in 76R MYC+ plants than *rmc* plants, respectively. There were no significant differences in plant B, Ca, Fe, Mg, N, Na and S concentrations (data not shown).

Table 3. Soil microbial biomass and mineral N pools ANOVA
GLM results given: n.s., not significant at $P < 0.05$, $n = 6$

	Plant	P Level	Plant × P level	Block
MBC	n.s.	n.s.	n.s.	n.s.
NH_4^+-N	n.s.	n.s.	n.s.	0.0006
NO_3^--N	<0.0001	n.s.	n.s.	n.s.

Discussion

Colonisation of roots by AMF did not have a measurable effect on cumulative or daily means of hourly soil respiration. Some earlier studies report an increase in respiration in roots colonised by AMF (Pang and Paul 1980; Peng *et al*. 1993), but others do not (Silsbury *et al*. 1983). Size asymmetry between mycorrhizal and non-mycorrhizal plants is an important factor. For example, using the same automated respiration monitoring system, Langley *et al*. (2005) reported higher respiration from *Helianthus annuus* L. plants colonised by AMF, an effect attributed to higher plant growth in the mycorrhizal treatments. Although no net effect of AMF on soil respiration was detected in this study, there may have been differences in root or mycorrhizal processes. Despite similar shoot and total plant biomass, root biomass and root length density were higher in *rmc* plants, which had essentially no (<1%) mycorrhizal colonisation. The specific root length of the 76R MYC+ roots was significantly higher (12.9 ± 0.3 cm/mg dry weight) than in the *rmc* plants (10.8 ± 0.7 cm/mg dry weight). Thus, the sum of root + AMF respiration (both autotrophic and heterotrophic

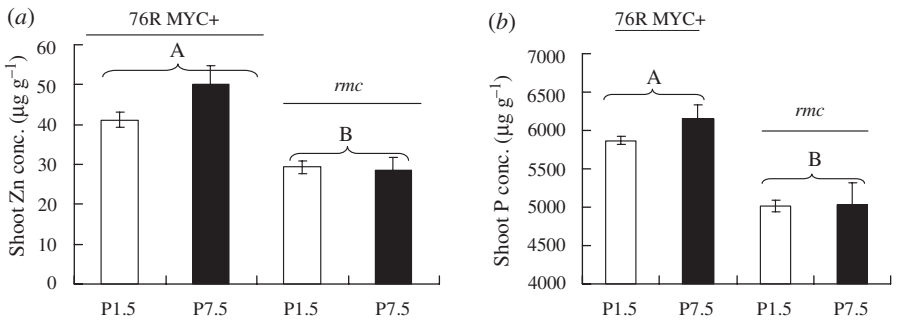

Fig. 4. (*a*) Shoot Zn concentration, and (*b*) shoot P concentration, of 76R MYC+ and *rmc* tomato plants grown in the low (P1.5) and high (P7.5) P addition treatments, respectively, i.e. 1.5 and 7.5 μg P added/g soil. Values are mean ± s.e., $n = 6$. Different letters indicate significant differences at $P < 0.05$ for genotype main effect.

respiration) in the 76R MYC+ treatment may have been equivalent to that of the more abundant, thicker but uncolonised roots in the *rmc* treatment. A lack of significant difference in cumulative respiration per unit root length density at the end of the experiment (data not shown) further supports the conclusion that the two genotypes did not differ in terms of net effects on soil respiration. Importantly, hourly respiration (average over the last 5 days of the experiment) per unit dry weight (at harvest) was higher in the 76R MYC+ (0.44 ± 0.05 mg C/g RDW) than *rmc* plants (0.27 ± 0.04 mg C/g RDW) (also compare Figs 2, 3*c*), indicating a significant effect of AMF colonisation on root respiration (see also Mortimer *et al*. 2005).

Mycorrhizal plants achieved higher uptake of P and Zn with similar soil respiration as non-mycorrhizal plants. There were no differences in tissue N concentrations here (shoot N <1% DW), although AMF can improve the N nutrition of plants (Marschner and Dell 1994; Ryan and Ash 1999; Cavagnaro *et al*. 2006). Although allocation of C to AMF can be <20% of a plant's photoassimilates (Jakobsen and Rosendahl 1990), these plants were not highly colonised (<20%), implying that these mycorrhizal plants may have allocated less C to AMF. The allocation of C to AMF may be approximately equal to the difference in root biomass C that was observed between the genotypes. Since the plant tissue contained 0.4 g C/g biomass (based on values for shoots and stems), then it follows that ~0.2 g more root C was present in the non-mycorrhizal plants. Based upon this value, we estimate that ~9% more C was present as root biomass in the non-mycorrhizal plants, given a total of 2.3 g C/plant in both genotypes. This simple estimation of C allocation to AMF, along with the finding that soil respiration is slightly higher per unit root biomass in mycorrhizal plants, and the assumption that AMF received <20% of photoassmilates (Jakobsen and Rosendahl 1990), suggests that approximately similar amounts of C may have been expended belowground in both genotypes. If so, then there was higher nutrient uptake per amount of C invested belowground, for construction, maintenance and functioning of AMF (e.g. see Wright *et al*. 1998) in the mycorrhizal plants. These calculations are indicative only, with more detailed modelling required before generalisations can be made. To this end, further studies of the type we report here, using soils with similarly low C levels, so as to minimise 'background' heterotrophic respiration, are required.

It is unlikely that differences in the wider soil biota affected soil respiration in the mycorrhizal and non-mycorrhizal treatments, mainly because MBC and total PLFA were similar in all treatments. This is not unexpected given that microbial biomass and total PLFA have been found to be highly correlated with each other (Potthoff *et al*. 2006). As in a previous study, PLFA analysis showed little difference in soil microbial communities between the two tomato genotypes (Cavagnaro *et al.* 2006). In that situation, the soil was from an organically-managed farm, and had higher MBC and the number of PLFA was also greater; 45 PLFA were present in >10% of the samples *v.* 29 PLFA in this study. Thus, the lack of effect was consistent across quite different soils. Using a DNA-based approach, shifts in the total bacterial community composition (Marschner and Timonen 2005), but not of the ammonia oxidising bacteria (Cavagnaro *et al*. 2007*a*), have been observed in the rhizosphere of these tomato genotypes and attributed to a complex series of interactions between genotype, AMF species and light levels.

In the absence of plants, the higher level of P addition to this soil resulted in lower cumulative soil respiration. A similar trend was observed in the treatments with plants, but was not significant. These results are difficult to explain but may be related to the availability of other ions. Phosphate can react with cations to give soluble molecular and ionic species as well as insoluble salts that may have affected microbial activity (Robertson and Alexander 1992). Previous studies have shown higher levels of soil respiration under low P conditions, probably due to increased C allocation to root production and activity (Keith *et al*. 1997; Nielsen *et al*. 1998), but at P concentrations much lower than this study.

The levels of extractable soil P in this study are considered below excessive levels for vegetables (Maynard and Hochmuth 1997). The leaf tissue levels were in the adequate to high range for this stage of growth in tomato, and the plants showed no visual symptoms of P toxicity (Maynard and Hochmuth 1997). Zinc levels were also in the adequate to high range, also indicating that P availability was not excessive, since overly high P levels can induce Zn deficiency in plants (Marschner 1995). High soil P can lead to a reduction in colonisation by AMF (Oliver *et al*. 1983; Baon *et al*. 1992), due to a reduction in fungal growth and/or to increases in the growth of roots (Smith 1982; Thomson *et al*. 1991; Bruce *et al*. 1994). Such effects were not seen here, or in a survey of organic tomato farms in California, where there was no correlation between mycorrhizal colonisation and extractable P (L. E. Jackson and L. A. Saxe, pers. comm.). Although plant available soil P was relatively high, it was considerably lower than in other studies where functional AMF were observed (e.g. Blanke *et al*. 2005). Further insight may be gained in future studies by taking into account effects of P on the metabolic activity of AMF (Ezawa *et al*. 2004; van Aarle *et al*. 2005; Valentine and Kleinert 2007), extra-radicle growth (Olsson *et al*. 2002; Cavagnaro *et al*. 2005) and the abundance of arbuscules or other structures (Cavagnaro *et al*. 2001; Jackson *et al*. 2002; Cavagnaro *et al*. 2003).

Conclusions

The formation of AM presents a complex series of tradeoffs between the C cost of the fungi and the benefits of enhanced nutrient supply to the plant (Johnson *et al*. 1997; Fitter *et al*. 2000). When colonised by AMF, a plant's nutrient demands can be met via the mycorrhizal pathway (Smith *et al*. 2004), often coupled with relatively lower root biomass (Cavagnaro *et al*. 2007*b*). That net soil CO_2 respired did not differ between the *rmc* and 76R MYC+ genotypes in this study, despite differences in root length and biomass between mycorrhizal treatments, indicates a trade-off between respiration associated with mycorrhizal fungi and that required to support non-mycorrhizal root function. Importantly, hourly respiration at the end of the experiment per unit root dry weight was higher in the 76R MYC+ than *rmc* plants. The genotypic approach to control for AM colonisation helped avoid underestimation of the heterotrophic component of soil respiration, and hence,

potential overestimation of the contribution of AMF to soil respiration. These data suggest that although net soil respiration may not differ between plants colonised by AMF and those not colonised, the mycorrhizal contribution to soil respiration may indeed be significant, and yet the nutrient gains per unit C expended belowground appears to be greater than in non-mycorrhizal plants.

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