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Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production

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Abstract

Effects of arbuscular mycorrhzal (AM) fungi on plant growth and nutrition are well-known, but their effects on the wider soil biota are less clear. This is in part due to difficulties with establishing appropriate non-mycorrhizal controls in the field. Here we present results of a field experiment using a new approach to overcome this problem. A previously well-characterized mycorrhizal defective tomato mutant (*rmc*) and its mycorrhizal wildtype progenitor (76R MYC+) were grown at an organic fresh market tomato farm (Yolo County, CA). At the time of planting, root in-growth cores amended with different levels of N and P, were installed between experimental plants to study localized effects of mycorrhizal and non-mycorrhizal tomato roots on soil ecology. Whilst fruit yield and vegetative production of the two genotypes were very similar at harvest, there were large positive effects of colonization of roots by AM fungi on plant nutrient contents, especially P and Zn. The presence of roots colonized by AM fungi also resulted in improved aggregate stability by increasing the fraction of small macroaggregates, but only when N was added. Effects on the wider soil community including nematodes, fungal biomass as indicated by ergosterol, microbial biomass C, and phospholipid fatty acid (PLFA) profiles were less pronounced. Taken together, these data show that AM fungi provide important ecosystem functions in terms of plant nutrition and aggregate stability, but that a change in this one functional group had only a small effect on the wider soil biota. This indicates a high degree of stability in soil communities of this organic farm.

Introduction

Ecological processes provide key ecosystem services for agriculture. Organically managed agroecosystems offer a unique context in which to study the ecological relationships between soil biology and biogeochemistry. Microbial processes are crucial for nutrient availability because only organic sources of nutrients can be used (USDA National Organic Program Standards). Organic farmers manage for high soil organic matter (SOM) and net N mineralization rates. Furthermore, use of synthetic pesticides, which can negatively impact soil biota (e.g., Miller and Jackson, 1997; Sukarno et al., 1993), is also prohibited (USDA National Organic Program Standards). As a consequence, microbial activity is often higher in organic than conventionally-managed soils (Carpenter-Boggs et al., 2000; Drinkwater et al., 1995; Lundquist et al., 1999), community composition can be more complex

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(Mäder et al., 2002), and in some instances, invertebrate density and biodiversity increases (Hole et al., 2005). Abundance of arbuscular mycorrhizal (AM) fungi may also increase under organic management (Mäder et al., 2002; Smukler and Jackson, personal communication).

AM fungi effectively increase the absorptive surface of the plant root system thereby providing access to soil-derived nutrients from sources not necessarily otherwise accessible to roots (Marschner and Dell, 1994; Smith and Read, 1997). The fungi benefit in return from a supply of plant-derived C (Barker et al., 1998; Harrison, 1999; Johnson et al., 1997). Although usually considered important primarily for P uptake, AM fungi can also increase both NH_4^+ and NO_3^- uptake (Frey and Schüepp, 1993; Johansen et al., 1993), and that of other nutrients including Zn, Cu, and K (Marschner and Dell, 1994). Such improvements in plant nutrition are of particular significance in soils of low nutrient status (Hetrick, 1991; Menge, 1983) and heterogeneous nutrient distribution (Cavagnaro et al., 2005; Tibbett, 2000), but may also be important for soils receiving frequent organic matter inputs as the primary source of nutrients, and those for which a complex soil food web regulates nutrient availability (de Deyn et al., 2001), such as organic farming systems.

The role of AM fungi in plant growth and nutrition, and as potential drivers of biodiversity and regulatory processes in soil ecosystems is not well understood. Direct and indirect effects on the soil biota may include altered plant exudation (Dixon et al., 1989; Graham et al., 1981), provisioning of high quality resources (hyphae and/or lipid rich spores) for soil grazers (Bakhtiar et al., 2001; Gange, 2000), localized changes in pH (Villegas and Fortin, 2001), and competition with other soil biota for soil resources (e.g., nutrients, water and space) (Koide, 1991; Tibbett, 2000). Such effects may help explain reported effects of AM fungi on rhizosphere bacterial community composition (Marschner et al., 2001; Paulitz and Linderman, 1989), and the wider soil community (Bakhtiar et al., 2001; Fitter and Sanders, 1992; Gange, 2000). Improvements in aggregate stability and soil structure due to AM fungi, mediated by entanglement and enmeshment mechanisms and secretion of stabilizing compounds by hyphae, have also been reported (Miller and Jastrow, 2000; Tisdall, 1991; Tisdall and Oades, 1979). However, in soils with high biological activity, large inputs of organic resources and intermittent (often seasonal) plant cover, the effects of AM fungi on soil biota and soil structure are unknown.

Attempts to study the ecology of AM *in situ* in the field, and to a lesser extent in pot studies, are complicated by the difficulties associated with establishment of appropriate non-mycorrhizal controls. This is largely due to non-specific effects of fumigants, fungicide or sterilization treatments used to establish such controls, or those that arise from the use of different plant species that are either constitutively mycorrhizal or non-mycorrhizal in a single experiment. Mycorrhiza defective plant mutants which have been used widely to study the developmental and molecular biology of AM (David-Schwartz et al., 2001; Duc et al., 1989; Gao et al., 2001; Gianinazzi-Pearson et al., 1991; and others), have potential for studies of AM ecology.

A tomato mutant with reduced mycorrhizal (fungal) colonization, named *rmc* (Barker et al., 1998) and its mycorrhizal wildtype progenitor (*L. esculentum* Mill. cv. 76R, refered to as 76R MYC+), have been well-characterized (Barker et al., 1998; Gao et al., 2001). Both genotypes have similar growth under non-mycorrhizal conditions (Cavagnaro et al., 2004; Poulsen et al., 2005), 76R MYC+ is responsive to colonization with AM fungi under various conditions (Cavagnaro et al., 2004; Poulson et al., 2005), and both genotypes grow well in field situations (Cavagnaro and Jackson, unpublished) and hence, have considerable potential for detailed studies of the functioning of AM in the field. We present results of a field study in which the tomato mutant *rmc* and its mycorrhizal wild-type progenitor, 76R MYC+, were grown on an organic tomato farm in central California. The aim was to study the ecology of AM in the field under on-farm organic management, when supplied with discrete patches of P and N, and their interactions with a complex microbial community. Specifically, the aims of this study were:

(1) Compare the growth and nutrition of a mycorrhiza defective tomato mutant and its mycorrhizal wild-type progenitor, grown under typical fresh market organic tomato production conditions;

(2) Measure the impact of roots colonized by AM fungi on the wider soil microbial and nematode communities; and
(3) Quantify the impact(s) of roots colonized by AM fungi on soil aggregation.

Material and methods

Site description

An experiment was established in a tomato field on an organically managed farm, Jim & Deborah Durst Farming, in Esparto, Yolo County, California. A tomato/cover crop/alfalfa rotation is employed and the farm has been organically managed since 1988. The soil is a Zamora loam, a fine-silty, mixed thermic, Mollic Haploxeralfs; soil properties are summarized in Table 1. A bed (1 m wide) and furrow system is employed on this farm, and the plants are sub-surface drip-irrigated. Lime is applied at the time of cover crop incorporation at a rate of 1122 kg/ha. Guano (10:10:2) is applied at a rate of 785 kg/ha (total) both prior to planting and as a side dressing during the growing season. Additional nutrients are supplied as a liquid fertilizer Agrolizer Liquid Fish Fertilizer (6-2-0; Natural Sciences Group, Inc., Fresno, CA) at a rate of 48 L/ha at the time of planting and again over the course of the growing season through the sub-surface drip tape. No composts, manures, or other organic amendments are added. The experiment was established at the same time the field was planted to commercial tomato production (May 20th, 2003).

Table 1. Soil characteristics of the experimental site in Central California, USA. Values are means ± SE, $n = 4$

Soil physical and chemical properties	Mean ± SE
Sand (%)	30 (4.3)
Silt (%)	41 (0.38)
Clay (%)	29 (0.29)
pH	6.8 (0.02)
N (Total) (%)	0.1 (0.004)
C (Total) (%)	0.8 (0.011)
C:N ratio	8.0 (0.4)
X-K (ppm)	199.5 (1.24)
X-K (meq/100 g)	0.5 (0.00)
X-Na (ppm)	98.9 (1.53)
X-Na (meq/100 g)	0.4 (0.00)
X-Ca (meq/100 g)	11.6 (0.03)
X-Mg (meq/100 g)	6.0 (0.08)
CEC (meq/100 g)	23.9 (0.48)
Zn (Total) (ppm)	82.1 (0.72)
Olsen-P (ppm)	18.2 (0.20)

Experimental design and planting

Pre-germinated seeds of the mycorrhiza defective tomato mutant (*rmc* hereafter), and its wildtype progenitor *L. esculentum* Mill. cv. 76R (see Barker et al., 1998) (76R MYC+ hereafter), were planted in peat moss in seedling trays and grown in a glasshouse for 4 weeks, followed by hardening in a lath house for 2 weeks. The transplants were used to establish an experiment (Randomized Complete Block Design) in a ca. 0.01 ha section of the field. There were three different treatments, each with two levels: Genotype (*rmc* or 76R MYC+), soil N addition (Low N or High N) and soil P addition (Low P or High P) (see below). All treatments were applied in combination, giving eight treatments, with a total of 32 plots across four blocks. Each block spanned eight beds in the field; each of the experimental plots (of which there were eight per block) contained seven plants, a buffer plant at either end of each plot, and five experimental plants. The seedlings were planted in rows (30 cm apart). In total there were 160 experimental plants and 64 buffer plants.

Root in-growth cores and nutrient addition

Effects of localized nutrient addition on mycorrhizas and their interactions with the wider soil biota were measured, using in-growth cores into which roots could readily grow, installed between experimental plants. At the time of planting, soil was collected at a depth of 15 cm in the area immediately surrounding the experimental plot. Eight days following collection, the soil (stored at room temperature) was passed through a 7 mm sieve and divided into four equal quantities. The different N and P treatments were applied to this soil as follows: Low N referred to as N0 hereafter (no added N) or High N referred to as N100 hereafter (100 μg N/g dry soil applied as $(NH_4)_2SO_4$), and Low P referred to as P0

hereafter (no added P) or High P referred to as P50 hereafter (50 μg P/g dry soil applied as $Ca(H_2PO_4)_2$). The N and P treatments were applied in combination; thus, the final nutrient treatments were: P0 + N0, P0 + N100, P50 + N0 and P50 + N100. Each treatment received 10 mL of the appropriate nutrient solution per root in-growth core. The following day, soil amended with the various nutrient treatments was packed into in-growth cores constructed of PVC pipes 102 mm in diameter and 52 mm in depth. Each in-growth core was packed with 505 g damp soil; a final bulk density of ca. 1.0 was achieved in the in-growth cores. Following soil packing, the ends of the root in-growth cores were covered with plastic mesh (1×2 mm openings).

Twenty-four hours following packing, the root in-growth cores were installed in the field. At the mid-point between each of the five central experimental plants in each plot, a hole was dug to expose the sub-surface drip irrigation line (ca. 20–22.5 cm below the soil surface), the hole was backfilled with ca. 25 mm of soil above the irrigation tape and the root in-growth core placed in the hole; mesh covered openings lay horizontal. To each root in-growth core, 62 mL of water was added at this time in order to achieve a similar moisture content as the surrounding soil. Soil was then packed between the sides of the in-growth core, and the hole was backfilled with the remaining soil. Four root in-growth cores were installed per plot giving 128 in-growth cores in total.

Plant sampling and sample analysis

Blocks 1 and 2 were harvested on the 30th July 2003 and Blocks 3 and 4 on the 1st August 2003. Aboveground plant biomass was harvested by cutting the plant stem at ground level. Plant samples were stored at 4 °C for ca. 3 days. Fruit and shoots were separated. Fruit fresh weights (FFW) were determined immediately, as were fruit dry weights (FDW) after drying at 60 °C. Shoot material was cut into ca. 200 mm segments, dried at 60 °C and shoot dry weights (SDW) determined. Following dry weight determination, plant material was ground. A composite sample of shoot material and a second composite sample of fruit material was prepared for each plot and used in nutrient analysis. Shoot and fruit Ca, Fe, K, Mg, Mn, Na, S, P and Zn contents were determined on plant material that was microwave-digested with nitric acid (Sah and Miller, 1992) and analyzed by ICP-AES (Thermo Jarrell Ash Corp., Franklin, MA, USA). Data are expressed as tissue nutrient concentrations. Stable isotope ratios of carbon and nitrogen were measured by continuous flow isotope ratio mass spectrometry (20–20 mass spectrometer, PDZEuropa, Northwich, UK) after sample combustion to CO_2 and N_2 at 1000 °C. The gases were separated on a Carbosieve G column (Supelco, Bellefonte, PA, USA) before introduction to the IRMS. Sample isotope ratios were compared to those of standard gases injected directly into the IRMS before and after the sample peaks and delta ^{15}N (DelAIR) and delta ^{13}C (DelPDB) values calculated. Final isotope values were adjusted to bring the mean values of standard samples distributed at intervals in each analytical run to the true values of the working standards. All N and C analyses were performed by the UC Davis Stable Isotope Facility (http://stableisotopefacility.ucdavis.edu/) in an on-line elemental analyzer (PDZEuropa ANCA-GSL).

Root in-growth core sampling and sample analysis

Immediately following harvest of the aboveground biomass, the root in-growth cores were carefully exposed, and any roots growing into and out of them cut, before removing the in-growth cores. The in-growth cores were then placed in plastic bags and immediately stored on ice in insulated containers. All soil samples were returned to the laboratory within 4 h of harvest and stored at 4 °C until soil sampling; all soil samples were processed within ca. 6 h of harvest. Due to the number of analyses made and the volume of the in-growth cores, pairs of adjacent cores were combined for all further analysis; consequently, there were two composite soil samples per experimental plot (64 samples in total). The soil samples were mixed by hand for 30 s after which uniform sub-samples were taken and soil analyses performed as outlined below.

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. 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). Separation, quantification, and identification of PLFA were done with a Hewlett Packard 6890 Gas Chromatograph (Bossio and Scow, 1998). Bacterial standards and software of the Microbial Identification System (Microbial ID, Inc.) were used to identify peaks. An internal standard (19:0) concentration was checked to obtain quantitative data in addition to the relative molar percentage of the molecules. Gas chromatography was also employed for some samples to validate peaks and provide greater resolution.

Duplicate sub-samples from each soil sample (25 g moist soil) were analyzed for microbial biomass carbon (MBC) by the fumigation extraction method (Vance et al., 1987). Triplicate soil samples (30 g moist soil) were taken, extracted with 2 *M* KCl, and inorganic N content determined colorimetrically using a modification of Miranda et al. (2001) for NO_3^- (plus NO_2^-) and Forster (1995) for NH_4^+. Gravimetric moisture was determined after drying approximately 50 g moist soil samples at 105 °C for 48 h.

A 20 g sub-sample of soil was taken for determination of ergosterol using a modification of the method reported by Djajakirana et al. (1996). Briefly, 2 g of fresh soil was mixed with 100 mL ethanol (200 proof, Gold Shield, Hayward, CA) and the foil-covered, sealed flask was incubated at room temperature for 30 min on a rotary shaker set at 250 rpm. All of the following steps were carried out either in darkness or in red light. The flask contents were vacuum filtered through a glass microfiber filter (Whatman GF/A) and the soil residue in the funnel was rinsed under vacuum with 3 aliquots of ethanol, 5 mL each. The filtrate was evaporated to dryness using a rotary evaporator (Büchi Roto-Vap) and a water bath at 60 °C. Preliminary data showed that ergosterol was fully stable if 60 °C was used for evaporation. The residue was reconstituted in 3 mL of 100% HPLC-grade methanol (Fisher Scientific, Pittsburgh, PA) and transferred into a 10 mL volumetric flask. This step was repeated twice more. Finally, the contents were brought up to volume (10 mL) with 100% methanol. The extracts were filtered through a 0.45 μm nylon membrane filter prior to injection into the HPLC system. The HPLC system consisted of a pump (Shimadzu Scientific Instruments, Inc., Columbia, MD; model LC−6A), a UV detector (Shimadzu; model SPD−10A), an injector (Rheodyne, Inc., Cotati, CA; model 7126), and an integrator/data system (Shimadzu; model CR501). A C_{18}, 0.46×15 cm column and a 2 cm guard cartridge (Spherisorb ODS−2, 5 μm, Alltech Associates, Deerfield, IL) were used to carry out the separations. Methanol (100%) was used as the mobile phase at a flow rate of 1 mL/min. The λ for detection was 282 nm. Ergosterol used as pure reference material was purchased from Sigma-Aldrich Chemical Co., St. Louis, MO.

Sub-samples of 200 g moist soil were taken for determination of root length density and colonization by AM fungi. Roots were extracted from soil by wet sieving. Root length was determined using a Comair root scanner (Commonwealth Aircraft Corp. Ltd., Melbourne, Australia). These roots were then cleared with 10% KOH (W/V) and stained with Trypan blue using a modification of the method of Phillips and Hayman (1970), omitting phenol from all reagents. Colonization of roots by AM fungi was then determined at 20× magnification using the gridline intersect method (Giovannetti and Mosse, 1980).

A soil sample of 200 g was taken for nematode community analysis. Nematodes were extracted using a combination of decanting and sieving and Baermann funnel methods (Barker, 1985). Nematodes were counted under a dissecting microscope, a sub-sample (minimum of 100) of which, were then identified at higher magnification to genus level. Nematode faunal analyses generated indicators of food web structure, status, functionality, and resource availability (Ferris et al., 2001; Ferris and Matute, 2003). The food web indices that were calculated were the Enrichment Index (EI), the Structure Index (SI) and the Channel Index (CI) as described by Ferris et al. (2001). Nematode biomass was calculated by applying the formula of Andrassy (1956) to the average dimensions of adults of each nematode taxon.

A 100 g sub-sample of soil was taken, and aggregates were separated by wet sieving as described by Elliott (1986). Prior to sieving, the soil was submerged for 5 min in deionized water. Four aggregate fractions were obtained:

>2000 μm (large macroaggregates), 250-2000 μm (small macroaggregates), 53-250 μm (microaggregates) and <53 μm (silt and clay fraction). All fractions were oven dried overnight (60 °C) and stored in closed glass jars at room temperature. Fraction weights were corrected for different sand contents before comparison.

The mean weight diameter (MWD) was calculated as:

$$\mathrm{MWD} = \frac{2000 + 8000}{2} \cdot [\mathrm{A}] + \frac{2000 + 250}{2} \cdot [\mathrm{B}] + \frac{250 + 53}{2} \cdot [\mathrm{C}] + \frac{53}{2} \cdot [\mathrm{D}]$$

where MWD is the mean weight diameter in μm, [A], [B], [C] and [D] are weight percentages of the large and small macroaggregates, microaggregates, and the silt and clay particles, respectively.

A composite soil sample across all treatments was made for each block. Soil particle size distribution, pH, CEC, exchangeable Na, exchangeable K, exchangeable Mg, exchangeable Ca, total N, total C and total Zn were determined by the DANR analytical laboratory, University of California Davis (http://danranlab.ucanr.org). Plant available (Olsen) P was also determined on the composite samples for each plot by the DANR Analytical Laboratory.

Statistical analysis

Plant and soil data were analyzed using GLM (see exceptions below) using SAS (version 8.02, SAS Institute, Cary, North Carolina). Prior to fitting of GLM, data normality was assessed in SAS using the Shapiro-Wilk W statistic (Zar, 1999). Non-normal data were log + 1, square root and Arcsin transformed and normality reassessed. Where transformation resulted in data normalization, GLM (see below) was performed on both the transformed and untransformed data. In all cases transformation had no effect on the outcome of GLM; thus, results from untransformed data are presented for ease of biological interpretation. Where significant effects were observed, pair-wise comparisons were made using Tukey's Test (Zar, 1999). All percentage data (colonization of roots by mycorrhizal fungi, aggregate sizes, nematode taxa as a percentage of total nematode community) were Arcsin transformed (Zar, 1999) prior to analysis.

Soil microbial community composition; PLFA analysis

Multivariate techniques were used to analyze the data for soil characteristics, management inputs, and PLFA data (as ng PLFA/g soil) using CANOCO, version 4.0 (Microcomputer Power, Inc., Ithaca, NY, USA). In graphical outputs, the position of the samples along the axes is determined by the loading scores, which describe the relative importance of a variable along the ordination axis. Genotypes and nutrient treatments types within a cluster on a graph are more similar to each other in terms of their sampled variables than other samples outside of that cluster. Only the 45 PLFA that were present in more than 10% of samples were included in the multivariate analyses. However, the total numbers of PLFA detected in each sample ranged between 25 and 47 distinct molecules/types. The relationship between microbial community composition, soil characteristics and experimental treatment (Genotype, P and N treatment) was analyzed by canonical correspondence analysis (CCA). The method constrains ordination axes into linear combinations of environmental variables, and will maximize the dispersion of the PLFA scores (ter Braak, 1987). Soil characteristics are represented by vectors. Vectors of greater magnitude and forming smaller angles with an ordination axis are more strongly correlated with that ordination axis. High scores of absolute value for a given PLFA or a given sample on a CCA axis indicate that it is highly related to the axis and to the environmental variable exhibiting high correlation to the axis. All soil characteristics and management variables were tested for significant contribution to the explanation of the variation in the PLFA data with the Monte Carlo permutation test associated with the forward selection subroutine in CANOCO. Only variables that were significant by the Monte Carlo permutation test at the $P<0.05$ level are included in the CCA biplot. Preliminary analysis identified four outliers in the CCA. All were samples from two adjacent plots in the field. None of the experimental variables were able to explain why these points were strong outliers, and thus these points were eliminated from the final analysis.

Results

Plant growth and nutrition

The two genotypes were closely matched in terms of fruit biomass (fresh and dry weights) and were unaffected by soil N and P additions in the root in-growth cores (Table 3). The vegetative dry weight was slightly higher (ca. 9% pooled over N and P addition treatments) in 76R MYC+ than *rmc* plants (Tables 2 and 3). There was also a significant three-way interaction between genotype, N and P for vegetative dry weight, which was higher in 76R MYC+ than *rmc* in the P0 + N100 nutrient addition treatment; all other differences were not significant. Root length (mean ± SE) in the in-growth cores was 2.3 ± 0.1 cm/g dry soil, and was not significantly affected by either genotype, N addition or P addition treatments, alone or in combination (Tables 2 and 4).

Shoot concentrations of P, Zn, N, S and Na were significantly higher in 76R MYC+ than *rmc* plants (Tables 2 and 3). Of particular note, the mean shoot P concentrations were ca. 1.5 times higher in 76R MYC+ than *rmc* plants (3810.3 and 2183.7 μg/g, respectively), as were those of Zn (64.9 and 42.3 μg/g, respectively). Conversely, shoot Mg, Mn and C concentrations were significantly lower in the mycorrhizal plants. The concentration of Mn in the shoots of 76R MYC+ plants were almost half those of *rmc* (98.4 and 180.7 μg/g, respectively). Nutrient concentrations in the fruit generally followed the same trend as those in the shoots. Fruit P and Zn concentrations were higher in 76R MYC+ than *rmc* plants, whereas concentrations of Mn were higher in *rmc*. In contrast to the shoots, the concentration of Na was significantly higher in the fruit of *rmc* plants.

Concentrations of Ca, P and S in the shoots were significantly greater in the N0 than N100 treatment across both genotypes (Tables 2 and 3). DelPDB was significantly higher (less negative) for both the shoots and fruit in the N100 treatment than the N0 treatment.

Soil nutrients and microbial biomass

Soil NH_4^+, at the end of the experiment, was significantly higher in the in-growth cores to which N had been added at the beginning of the experiment than in other treatments (Tables 2 and 4). This was not true of NO_3^-. Soil NO_3^-, however, was higher in in-growth cores containing roots of *rmc* than 76R MYC+ plants. Soil P was also significantly affected by P addition and genotype; soil from in-growth cores containing *rmc* roots had higher P than those containing 76R MYC+ roots (Tables 2 and 4).

There was a significant effect of genotype on colonization of roots by mycorrhizal fungi (Arcsine transformed), with that of *rmc* and 76R MYC+ being 4.7 and 24.2%, respectively. Both soil ergosterol and MBC were not significantly affected by genotype, N addition or P addition alone or in combination (Tables 2 and 4).

Soil aggregates

The mean weight diameter (MWD) of aggregates across the experiment was 0.50 (±0.12) mm and was unaffected by any treatment (Table 5). The percentages of aggregates in the size classes >2000, 250–2000, 53–250 and <53 μm, are given in Table 5. Means (±SE) for the different size classes, pooled over all treatments, were 1.8% (±0.1), 28.8% (±1.0), 55.7% (±1.0) and 16.3% (±0.3) in the >2000, 250–2000, 53–250 and <53 μm size classes, respectively. As only 1.8% (±0.1) of aggregates were >2000 μm fraction, we focus on the 250–2000 μm fraction, in which there was a significant interaction between genotype and N addition (Table 2). The fraction of small macroaggregates (250–2000 μm) in soil from in-growth cores (irrespective of P treatment) containing roots of 76R MYC+ plants at low N (N0) were significantly greater than those of the 76R MYC+ plants with additional N (N100), but not those from *rmc* at either N level. All other differences for this fraction were not significant (Tables 2 and 5). The greatest difference in the fraction of small macroaggregates was observed between the N0 and N100 treatments when 50 μg P/g dry soil was added to the 76R MYC+ plants; higher N reduced small macroaggregates from 35 to 23% (Table 5).

Nematodes

Nematodes representing 12 different taxa were identified in this soil; however, total numbers of

Table 2. ANOVA results for all treatments and response variables where significant differences were revealed. Plant (*L. esculentum*) genotypes were *rmc* or 76R MYC+. StDel PDB refers to shoots and FDel PDB refers to fruits

	G	P	N	G*P	G*N	N*P	G*N*P
Biomass (g)							
SDW	*						**
Shoots (μg/g)							
Ca			**				
Mg	***						
Mn	***						
Na	**						
P	***		*				
S	***		**				
Zn	***						
N	***						
C	*						
Fruit (μg/g)							
Mn	*						
Na	***						
P	***						
Zn	**						
Natural abundance							
StDelPDB			*				
FDelPDB			**				
Plant or soil properties							
AM colonization	***				*		
NH_4^+ (μg N/g dry soil)			*				
NO_3^- (μg N/g dry soil)	*						
Olsen-P (μg P/g dry soil)	*	***					
Size classes (μm)							
% >2000						*	
% 250–2000			**		*		
% 53–200						**	
% <53			**			***	
MWD (mm)							
Nematode groups							
Acrobeloides		*					
Aphelenchus		*					
Tylenchorhynchus		*					
Tylenchidae		**				*	
Fungal feeder biomass		**					
Plant feeder biomass		*				*	

Treatments are: G, Genotype; P, P addition treatment; N, N addition treatment and all combinations therein. *P < 0.05, **P < 0.01, ***P < 0.0001.

nematodes were relatively low (mean = 107.4 ± 5.7 individuals 100 g dry soil^{-1}) (Tables 2 and 6). In terms of biomass, but not numbers (data not shown), nematodes belonging to the taxa Acrobeloides, Aphelenchus, Tylenchorhynchus, Tylenchidae, as well as biomass of fungal-feeding and plant-feeding nematodes, all increased in response to P addition. There were no significant effects of any of the experimental treatments on the numbers of fungal-feeding, bacterial feeding or total

Table 3. Shoot dry weight (SDW), fruit fresh weight (FFW), fruit dry weight (FDW), nutrient concentrations, DelPDB, and DelAir of 76R MYC+ and *rmc* genotypes (*L. esculentum*), at different concentrations of N (N0 = 0 μg N/g dry soil, N100 = 100 μg N/g dry soil) and P (P0 = 0 μg P/g dry soil, P50 = 50 μg P/g dry soils) added to in-growth cores, at harvest

rmc					76R MYC+			
	P0+N0	P0+N100	P50+N0	P50+N100	P0+N0	P0+N100	P50+N0	P50+N100
Biomass (g)								
FFW	565 (77)	528 (148)	609 (114)	539 (102)	571 (60)	642 (81)	414 (42)	525 (53)
FDW	17 (2)	17 (4)	22 (5)	18 (5)	18 (3)	18 (2)	14 (1)	15 (2)
SDW	197 (10)	159 (8)	178 (10)	180 (11)	189 (12)	215 (11)	197 (11)	183 (14)
Shoots (µg/g)								
Ca	43997 (1397)	41660 (779)	43242 (719)	42628 (1591)	42824 (1407)	41067 (1120)	41766 (1261)	37554 (2681)
Fe	462 (96)	298 (77)	349 (69)	286 (31)	328 (29)	481 (103)	379 (30)	262 (25)
K	25158 (1644)	23788 (182)	23073 (740)	23905 (1277)	25661 (654)	24953 (1204)	26126 (430)	22055 (1515)
Mg	13639 (421)	14066 (33)	13701 (352)	13697 (244)	11902 (211)	11895 (375)	11610 (165)	10974 (856)
Mn	178 (11)	187 (7)	187 (6)	172 (10)	96 (4)	101 (4)	97 (6)	100 (14)
Na	712 (25)	663 (55)	668 (37)	713 (42)	781 (19)	837 (49)	742 (6)	742 (20)
P	2368 (253)	2162 (150)	2054 (162)	2151 (293)	4084 (235)	3876 (177)	4118 (181)	3163 (199)
S	9046 (542)	8337 (187)	8882 (314)	8408 (166)	11137 (192)	10183 (586)	11067 (132)	9361 (605)
Zn	41 (3)	44 (0.1)	43 (2)	42 (4)	65 (1)	77 (12)	64 (1)	55 (4)
N	29766 (1234)	29958 (1483)	29425 (1112)	29821 (1167)	32770 (1001)	32992 (1378)	34733 (939)	33124 (428)
[a]C	368 (2)	368 (1)	364 (4)	368 (2)	363 (2)	364 (4)	360 (3)	361 (3)
Fruit (µg/g)								
Ca	1692 (87)	1567 (67)	1772 (286)	3525 (1884)	1556 (192)	1809 (180)	1590 (274)	1695 (290)
Fe	60 (35)	14 (14)	23 (4)	32 (18)	28 (12)	104 (73)	137 (120)	17 (11)
K	62725 (2109)	61290 (1408)	62744 (3452)	62156 (3987)	61277 (4063)	61318 (3110)	62210 (1871)	61911 (2176)
Mg	2711 (153)	2590 (105)	2778 (272)	2934 (271)	2663 (259)	2762 (210)	2771 (158)	2711 (168)
Mn	20 (1)	20 (2)	21 (3)	26 (5)	16 (2)	19 (1)	19 (3)	17 (2)
Na	893 (103)	829 (40)	1006 (236)	811 (179)	541 (41)	652 (46)	543 (24)	585 (60)
P	7854 (626)	7010 (384)	7423 (588)	7314 (1099)	10346 (904)	10272 (646)	10593 (356)	10404 (514)
S	2116 (135)	2045 (192)	2271 (258)	2256 (185)	2132 (127)	2068 (113)	2214 (76)	2319 (141)
Zn	43 (2)	42 (4)	48 (5)	42 (5)	53 (5)	53 (4)	56 (6)	56 (3)
N	49368 (664)	44701 (4140)	49078 (1031)	47640 (1922)	47460 (2562)	49620 (1020)	51643 (1331)	50812 (495)
[a]C	446 (8)	405 (3)	442 (4)	452 (6)	41 (23)	439 (5)	425 (8)	437 (9)
Natural abundance								
StDelAir	5.3 (0.2)	5.2 (0.1)	5.2 (0.3)	5.0 (0.2)	5.2 (0.1)	5.5 (0.4)	5.1 (0.5)	5.3 (0.2)
StDelPDB	−28.4 (0.1)	−28.3 (0.1)	−28.5 (0.1)	−28.3 (0.0)	−28.6 (0.1)	−28.3 (0.2)	−28.4 (0.1)	−28.3 (0.0)
FDelAir	9.1 (0.4)	8.8 (0.3)	8.7 (0.7)	8.6 (0.9)	8.6 (1.0)	8.7 (0.6)	8.4 (1.0)	8.5 (0.8)
FDelPDB	−27.2 (0.2)	−27.2 (0.1)	−27.5 (0.1)	−27.1 (0.2)	−27.5 (0.1)	−27.3 (0.0)	−27.4 (0.1)	−27.0 (0.1)

Values are means ± SE, n = 4. Nutrient concentrations in tissues are μg/g except those marked with an [a] which are mg/g.

nematodes, nor on the calculated food web indices (data not shown).

Soil microbial community composition; PLFA analysis

Using canonical correlation analysis (CCA), we identified the factors that best explain the pattern of PLFA profiles via Monte Carlo tests (Figure 1). Only soil moisture and ammonium concentration significantly influenced the soil microbial community composition. None of the discrete experimental treatments (P and N addition treatment or Genotype) had a significant effect on the soil microbial community composition. This was evident in the CCA biplot, in which there was no clustering of samples from a given treatment (Figure 1).

Table 4. Root length, arbuscular mycorrhizal (AM) fungal colonization (%), MBC, ergosterol, inorganic N, and plant available-P (Olsen) in in-growth cores

Plant or soil properties	*rmc*				76R MYC+			
	P0+N0	P0+N100	P50+N0	P50+N100	P0+N0	P0+N100	P50+N0	P50+N100
Root length (cm/g dry soil)	2.5 (0.2)	2.3 (0.2)	2.2 (0.3)	3.0 (0.4)	2.4 (0.3)	1.9 (0.2)	2.4 (0.2)	2.0 (0.2)
[a]AM colonization	6.8 (2.5)	3.1 (1.0)	5.4 (1.8)	3.4 (0.6)	22.5 (3.6)	29.5 (4.3)	19.4 (1.2)	25.4 (3.1)
MBC (μg/g dry soil)	183.3 (11.2)	187.5 (12.7)	171.0 (13.4)	172.2 (12.6)	154.2 (11.9)	197.2 (14.3)	164.4 (12.7)	169.4 (12.6)
Ergosterol (μg/g dry soil)	890.2 (29.8)	1007.3 (77.0)	997.7 (25.8)	1008.0 (26.2)	928.7 (64.6)	875.0 (33.4)	926.5 (45.5)	972.0 (35.3)
NH_4^+ (μg N/g dry soil)	4.8 (2.8)	6.0 (3.0)	4.5 (2.4)	7.0 (4.4)	1.9 (0.5)	9.4 (4.5)	2.9 (0.8)	6.5 (3.8)
NO_3^- (μg N/g dry soil)	6.1 (1.5)	7.0 (1.2)	6.6 (1.4)	5.8 (1.4)	3.6 (0.6)	6.2 (1.6)	4.1 (0.6)	5.8 (1.2)
Olsen-P (μg P/g dry soil)	15.3 (0.6)	16.6 (0.3)	20.9 (1.5)	20.1 (0.4)	14.7 (0.7)	14.4 (0.6)	19.7 (0.9)	19.2 (0.9)

Treatments are: G, Genotype; P, P addition treatment; N, N addition treatment and all combinations therein. *rmc* or 76R MYC+ genotypes of *L. esculentum*, N (N0 = 0 μg N/g dry soil, N100 = 100 μg N/g dry soil) and P (P0 = 0 μg P/g dry soil, P50 = 50 μg P/g dry soil). Values are means ± SE, $n = 8$.
[a]AM fungal colonization is Arcsine transformed.

Table 5. Percentages of aggregates in a given size class and MWD (Mean Weight Diameter) in in-growth cores

Size classes (μm)	*rmc*				76R MYC+			
	P0+N0	P0+N100	P50+N0	P50+N100	P0+N0	P0+N100	P50+N0	P50+N100
>2000	1.8 (0.5)	1.6 (0.3)	1.8 (0.3)	1.7 (0.3)	2.2 (0.4)	1.8 (0.3)	1.4 (0.2)	2.7 (0.5)
250–2000	28.7 (2.1)	29.5 (2.8)	30.7 (2.4)	26.5 (3.3)	30.9 (3.3)	26.3 (2.0)	34.6 (2.9)	22.8 (0.25)
53–200	57.5 (2.6)	53.6 (2.7)	52.4 (2.5)	57.9 (3.1)	56.9 (4.2)	56.5 (2.2)	50.1 (2.5)	60.7 (2.6)
<53	15.3 (0.5)	18.0 (1.0)	17.4 (0.6)	15.9 (0.8)	13.3 (0.4)	18.4 (0.5)	15.7 (0.7)	16.3 (0.8)
MWD (mm)	0.49 (0.03)	0.49 (0.03)	0.51 (0.03)	0.46 (0.04)	0.54 (0.05)	0.47 (0.03)	50.3 (0.03)	0.48 (0.05)

Values are means ± SE, $n = 8$. *rmc* or 76R MYC+ genotypes of *L. esculentum*, N (N0 = 0 μg N/g dry soil, N100 = 100 μg N/g dry soil) and P (P0 = 0 μg P/g dry soil, P50 = 50 μg P/g dry soil). Percentages of aggregates in a given size class are Arcsine transformed.

PLFA (followed by loading score) that had a close association with Axis 1 included 17:1 ω7c (1.1438), 18:3 ω6c (0.3608), 19:0 ant (0.289) and 13:0 iso (0.253); their loading scores were positive on this axis. None showed a clear pattern with regard to any of the environmental parameters that were measured. The PLFA (followed by loading scores) identified as 17:0 2OH (−0.5243) and 18:1 ω7t (−0.316) were found to have a close association with Axis 2; their loading scores on this axis were negative. Axis 2 explained considerably less of the variation in the data than Axis 1 (11.2 vs. 37.8%, respectively). As with Axis 1, factors influencing these PLFA were not revealed in the CCA. The PLFA 18:1 ω7t has been associated with gram-negative bacteria (Hurst et al., 1996), *Psuedomonas putida* (Heipieper et al., 1992) and methane oxidizing bacteria (Nichols et al., 1985). The PLFA 16:1 ω5c did not have an important loading score in this analysis.

Discussion

Differential colonization of the *rmc* and 76R MYC+ tomato genotypes by AM fungi, coupled with their near-matched growth (in this study and in a previous pot studies when grown in the absence of AM fungi e.g., Cavagnaro et al., 2004), provides a model system to study the ecology of AM in the field. AM fungi clearly play an important role in the nutrition of organically grown tomatoes and may help to retain nutrients in these ecosystems. Colonization of tomato roots by AM fungi had little effect, however, on the wider soil community in this study. This may

Table 6. Biomass (μg) of fungal-feeding, bacterial feeding and total nematodes, and specific nematode taxa/100 g dry soil

Nematode groups	*rmc*				76R MYC+			
	P0+N0	P0+N100	P50+N0	P50+N100	P0+N0	P0+N100	P50+N0	P50+N100
Panagrolaimus	2.0 (1.4)	2.9 (1.0)	2.3 (0.9)	3.0 (1.3)	1.1 (0.4)	1.3 (0.4)	1.9 (0.9)	1.5 (0.9)
Rhabditidae	91.2 (27.8)	96.6 (23.4)	93.4 (33.5)	86.4 (22.3)	107.3 (52.7)	28.7 (6.2)	100.0 (43.8)	83.9 (51.8)
Acrobeloides	1.2 (0.4)	1.2 (0.2)	2.0 (0.6)	1.6 (0.3)	0.9 (0.2)	1.3 (0.2)	1.2 (0.3)	2.1 (0.5)
Plectus	1.7 (1.7)	0.3 (0.3)	0.0 (0.0)	0.0 (0.0)	0.1 (0.1)	0.4 (0.4)	1.0 (0.7)	0.9 (0.5)
Prismatolaimus	0.2 (0.2)	0.6 (0.4)	1.2 (0.8)	2.3 (1.6)	0.2 (0.2)	0.6 (0.4)	1.2 (1.1)	0.8 (0.5)
Aphelenchus	7.0 (1.8)	9.2 (1.9)	11.6 (2.8)	10.7 (2.2)	5.5 (0.9)	7.2 (1.7)	8.3 (1.6)	12.4 (2.3)
Other Dorylaims	728.7 (508.3)	949.2 (216.0)	1372.3 (562.8)	615.4 (237.7)	761.6 (228.6)	1305.6 (288.5)	577.3 (190.7)	900.4 (318.1)
Discolaimus	2.4 (1.2)	3.4 (1.4)	1.6 (1.6)	3.3 (2.1)	3.6 (2.2)	2.0 (1.3)	0.6 (0.6)	0.0 (0.0)
Pratylenchus	0.5 (0.2)	0.3 (0.1)	0.3 (0.1)	0.3 (0.1)	0.4 (0.1)	0.4 (0.2)	0.3 (0.1)	0.5 (0.1)
Tylenchorhynchus	3.4 (0.9)	2.5 (0.5)	3.6 (0.8)	4.0 (0.7)	2.4 (0.3)	2.6 (0.4)	2.9 (0.5)	5.9 (1.5)
Xiphinema	2.5 (2.5)	3.0 (2.1)	7.0 (4.6)	3.3 (2.2)	1.1 (1.1)	0.0 (0.0)	4.7 (4.7)	0.0 (0.0)
Tylenchidae	3.7 (0.3)	2.9 (0.4)	4.8 (1.0)	6.3 (0.9)	3.9 (0.5)	4.1 (0.9)	3.9 (0.3)	6.5 (1.2)
Total nematode biomass	844.5 (538.6)	1072.2 (216.3)	1500.1 (599.2)	736.5 (240.7)	888.2 (248.0)	1354.2 (290.7)	703.3 (199.7)	1014.8 (305.4)
Fungal feeder biomass	8.9 (1.9)	10.7 (2.1)	14.0 (3.3)	13.8 (2.5)	7.5 (1.0)	9.2 (2.0)	10.3 (1.7)	15.6 (2.7)
Bacteria feeder biomass	96.3 (28.4)	101.6 (23.8)	98.9 (34.4)	93.3 (22.9)	109.6 (52.6)	32.3 (6.5)	105.4 (43.3)	89.2 (52.7)
Omnivore biomass	731.1 (509.3)	952.6 (216.0)	1373.9 (562.5)	618.6 (238.2)	765.3 (229.1)	1307.5 (287.6)	577.9 (190.7)	900.4 (318.1)
Plant feeder biomass	2.3 (0.3)	1.7 (0.3)	2.7 (0.6)	3.5 (0.4)	2.3 (0.2)	2.5 (0.6)	2.2 (0.2)	3.7 (0.6)

rmc or 76R MYC+ genotypes of *L. esculentum*, N (N0 = 0 μg N/g dry soil, N100 = 100 μg N/g dry soil) and P (P0 = 0 μg P/g dry soil, P50 = 50 μg P/g dry soil). Values are means ± SE, n = 8.

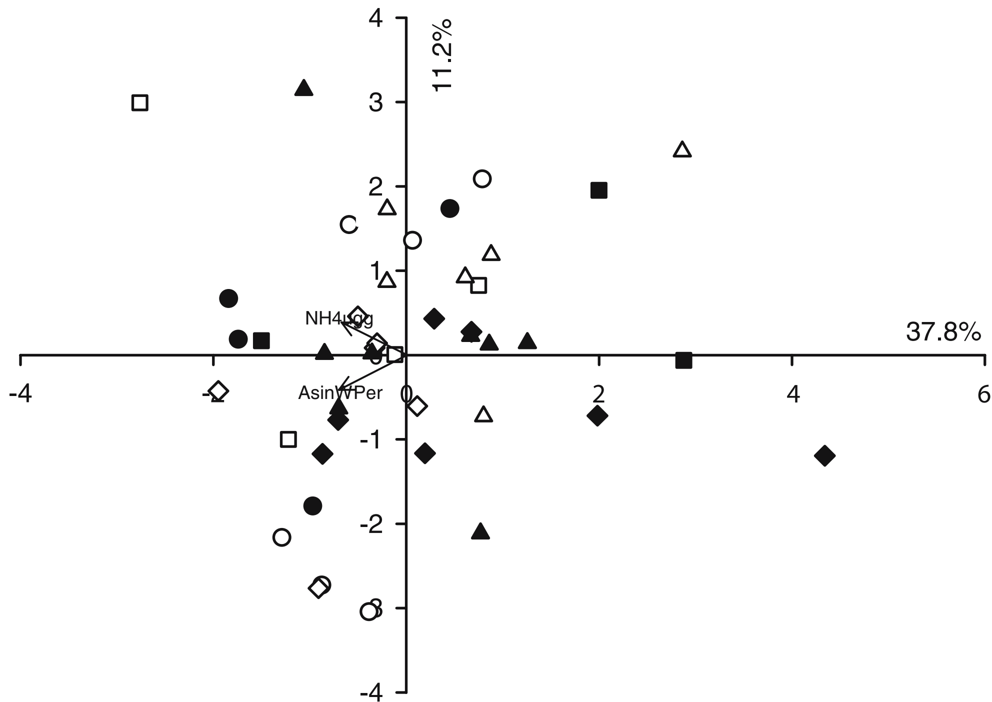

Figure 1. CCA ordination biplot of PLFA profiles of microbial communities in soil from in-growth cores, which are classified by experimental treatments: ●, P0 + N0 *rmc*; ○, P0 + N0 76R MYC +; ■, P0 + N100 *rmc*; □, P0 + N100 76R MYC +; ▲, P50 + N0 *rmc*; Δ, P50 + N0 76R MYC +; ♦, P50 + N100 *rmc*; ◇, P50 + N100 76R MYC +. Vectors represent the soil characteristics from in-growth cores that are significant by the Monte Carlo test ($P < 0.05$). Vectors are: NH_4 μg g, soil N–NH_4^+ concentration; ArcsineWper, Arcsine transformed soil moisture content (%). Axes 1 and 2 represent 37.8 and 11.2% of the variation in the data, respectively. For loading scores of individual PLFA, see text.

be a reflection of the biocomplexity inherent in these ecosystems, and an indication of a high degree of stability and possibly resilience in community composition and structure in an organic farming system.

Plant growth and nutrition

The aboveground (vegetative and fruit) biomass of the *rmc* and 76R MYC + tomato genotypes was closely matched at harvest, but there were effects on fruit nutritional quality (see below). Near matched growth of these genotypes in the field avoided confounding effects associated with size asymmetry and its resulting effects on plant nutrition and soil ecology. It is anticipated that there may be growth differences early in the establishment of the association, when C is allocated to the AM fungi rather than plant (root and/or shoot) biomass (Johnson et al., 1997), and before the initial "C investment" in the AM fungi is compensated for by increased soil resource exploitation, provisioning (Cavagnaro et al., 2005; Facelli and Facelli, 2002; Johansen et al., 1993; Ryan and Angus, 2003; Tibbet, 2000) and/or enhanced disease resistance (Perrin, 1990). Although reductions in root length in mycorrhizal plants have been reported (Smith and Read, 1997), our lack of differences most likely reflect the small volume of the in-growth cores, and their placement in a zone (above the sub-surface irrigation line) of intense root growth.

The two genotypes were differentially colonized, consistent with previous pot studies using known AM fungi (see Gao et al., 2001 for detailed characterization). The *rmc* roots had some surface colonization (4.7%); however, colonization was incomplete and did not proceed beyond the epidermis. Conversely, the 76R MYC + plants were 24.2% colonized with the formation of appresoria, intercellular hyphae, arbuscules, hyphal coils, arbusculate coils and vesicles.

Colonization of tomato roots by AM fungi in California fresh market organic tomato farms are typically in the range of 7–37% (Jackson and Saxe, personal communication). The fact that the genotypes were differentially colonized, and that the association between 76R MYC+ and the indigenous AM fungi were clearly functional in terms of nutrient uptake (see below), strengthens the utility of these genotypes to study the ecology of roots colonized by AM fungi in this field context.

Concentrations of nutrients in both fruits and shoots generally followed similar trends. The shoot and fruit P concentrations were ca. 75 and 41% higher in colonized 76R MYC+ than non-mycorrhizal *rmc* plants, respectively. This contrasts with an earlier pot study of the 76R MYC+ genotype (Burleigh et al., 2002), in which inoculation with seven different AM fungi (individually) relative to uninoculated controls, did not significantly affect shoot P concentrations. The plants in that study were, however, harvested prior to fruit development and there were differences in vegetative biomass (Burleigh et al., 2002). Of particular interest here are the ca. 54 and 24% increases in shoot and fruit Zn concentrations, respectively, of the 76R MYC+ plants relative to *rmc* plants. Previous studies have estimated that 25% of the Zn uptake by plants can be supplied by AM fungi (Marschner and Dell, 1994). Zinc has been identified as a key human mineral nutrient deficiency (Welch and Graham, 2004); our results indicate that colonization of roots by AM fungi can significantly improve the nutritive value of foods under some circumstances.

76R MYC+ plants had higher shoot N concentrations (ca. 12.3%) than their non-mycorrhizal *rmc* counterparts. The concentration of NO_3^- at harvest in soil from in-growth cores containing roots of 76R MYC+ was also significantly lower than those containing *rmc* roots. Taken together, these results suggest that AM fungi not only play an important role in plant N nutrition, but may also help mitigate ecosystem N losses. This is especially important in agroecosystems where timing of inorganic N release and crop N uptake are not always synchronized (Robertson et al., 1997). Similarly, root length differences between cover crop species will also be important. Similarly, plant P concentrations were higher and soil P concentrations lower, in the 76R MYC+ vs. *rmc* treatments, respectively. In the case of both N and P, this is likely related to an enhanced foraging ability and capture of nutrients by mycorrhizal root systems (Olsson et al., 2002; St John et al., 1983; Tibbet, 2000).

Concentrations of Mn in the shoots and fruit, and Mg in the shoots, were significantly higher in *rmc* than 76R MYC+ plants. Decreased concentrations of Mn in the shoots and roots of mycorrhizal plants have been demonstrated previously, and attributed to increased population sizes of manganese oxidizers in the rhizosphere of colonized plants (Arines et al., 1989; Kothari et al., 1991; Marschner, 1995). Mg uptake can be competitively inhibited by other soil cations including K^+, Ca^+ and especially Mn^{++}, although given the lower Mn concentration in the mycorrhizal plants, this is unlikely. Mg may also become limited in drought stressed plants; although there were no indications of one genotype being more drought-stressed than the other (see Delta ^{13}C values, Table 3). Also of note were the lower and higher concentrations of Na in the fruits and shoots of 76R MYC+ plants, respectively, relative to the *rmc* plants, which have no clear explanation.

Despite the small volume of the in-growth cores, addition of N resulted in significant effects on whole-plant responses. Concentrations of Ca, P and S in the shoots were significantly greater in the N0 than N100 treatment. This may reflect greater foraging by the hyphae under lower N concentration and subsequent uptake of other nutrients from the soil within the in-growth cores. An alternative explanation is that higher N uptake stimulated the uptake of other limiting nutrients by roots from throughout the root zone. The large root-foraging response to a small patch of higher soil N suggests that roots acclimated by increased N absorption rates rather than increased root length within the patch (Jackson et al., 1990) to increase N uptake. Higher N availability in the patches caused DelPDB to be significantly higher (less negative) for both the shoots and fruit, suggesting higher photosynthetic capacity and/or greater isotopic discrimination during periods of carbon gain and/or lower intrinsic water use efficiency (Lambers et al., 1998; Morgan, 1986; Shangguan et al., 2000).

Ergosterol, microbial biomass and PLFA

Despite differences in mycorrhizal fungus colonization between 76R MYC+ and *rmc* plants, there were no differences in either microbial biomass (MBC) or the fungal marker, ergosterol. The ubiquity and presence of ergosterol in AM fungi has been debated recently (Hart and Reader, 2003a, b; Olsson et al., 2003). Our data support the observations of Olsson et al. (2003) that ergosterol is not necessarily correlated with AM fungal biomass (inferred here via differential colonization of tomato roots). The validity of using ergosterol as a biomarker for fungi needs to be further explored in a wide range of settings and through comparisons of soils in which the types and abundances of fungi differ.

Phospholipid fatty acids are present in the membranes of all living cells and are rapidly turned over following cell death, making them excellent signature molecules for the live microbial biomass (Bossio and Scow, 1998). Although PLFA does not identify specific organisms, it shows major groupings, is rapid, has no PCR bias, is quantitative and is useful in relating changes in the total microbial community to environmental parameters (Green and Scow, 2000). Soil moisture and soil NH_4^+ concentration significantly influenced the soil microbial community composition, as found previously, in response of agricultural soils to irrigation (Bossio et al., 1998; Lundquist et al., 1999; Steenwerth et al., 2002) and soil N (Bossio et al., 1998; Steenwerth et al., 2002), although results are not always consistent (Bossio et al., 1998). Varied responses in PLFA profiles (Olsson et al., 1996), as well as changes in rhizosphere bacterial community composition using DNA-based methods (Johansson et al., 2004; Marschner and Baumann, 2003; Wamberg, et al., 2003) in the presence of AM fungi have been reported. However, no such effects were observed in our study. This may be attributed to the preponderance of non-rhizosphere soil sampled in the present study.

None of the specific PLFA with high loading scores could be explained by the measured environmental parameters, and thus, may be associated with variables not measured here. The reported AM fungal marker 16:1 ω5c was not an important loading score in this analysis, and thus does not appear to have high specificity as an AM fungal marker in this soil. This PLFA is also present in many gram-negative bacteria and type 1 methanotrophs (Nichols et al., 1985; Olsson et al., 1995); therefore, its use as a specific indicator of AM fungi in soil (not roots), needs to be considered carefully.

Soil aggregates

This soil was not well aggregated, with few macroaggregates and a relatively low MWD, consistent with the low total soil C (0.8%) and history of frequent disturbance by tillage. The presence of roots colonized by AM fungi and nutrient addition had a significant effect on soil aggregation over the short time period of this experiment. Foraging activities of hyphae may have been greater in the lower N soil treatment, resulting directly or indirectly in increased aggregate stability. The percentage of small macroaggregates was not correlated with microbial biomass (MBC), fungal biomass as indicated by ergosterol or number of total, fungal-feeding or bacterial feeding nematodes, suggesting that hyphae of AM fungi are likely the primary agent responsible for the increase in aggregate stability. This is also consistent with the observed higher hyphal length density associated with 76R MY plants than *rmc* plants in a pot study (Poulsen et al., 2005).

Nematodes

Nematode communities were apparently not strongly impacted by the presence of roots colonized by AM fungi. This may be related to the palatability of AM fungi, niche overlap with other soil fungi, competitive exclusion of other hyphae by the AM fungi, or AM fungi representing a small fraction of the fungal biomass in the soil. In a frequently disturbed ecosystem, spores may dominate AM fungal inoculum sources rather than intact hyphal networks (due to disruption of hyphae), thus a change in AM fungal hyphal length may not have a large impact on fungal feeding nematodes (e.g., Baktiar et al., 2001). The lack of correlations between ergosterol and the biomass or numbers of fungal feeding nematodes, fungal-feeding and bacterial

feeding nematodes, bacterial feeding nematodes and MBC, and fungal feeding nematodes and MBC, substantiates that nematode communities were insensitive to changes in the microbial parameters measured here, at least at the time and spatial scale of our study.

Addition of P increased biomass but not numbers of specific groups of nematodes. Previous studies have shown differential responses of nematodes to inputs of inorganic N (Tenuta and Ferris, 2004) and similar processes may be triggered by P addition. It is possible the effect is indirect with P affecting other trophic levels within the soil food web that in turn impact nematode biomass. The lack of agreement between biomass and numbers of nematodes highlights the complexity associated with quantifying changes in soil communities. Biomass calculations are made from the average dimensions of adult nematodes, and do not take into account the size distribution across developmental stages.

Conclusions

AMF played an important role in plant nutrition, and to a lesser extent, in soil structure at this organic farm. The higher Zn content in the fruit of mycorhizal plants is of interest for human nutrition. This experimental system eliminates confounding factors from non-specific fumigation or resulting from the use of constitutively mycorrhizal and non-mycorrhizal plant species, and indicated that AM fungi did not have much impact on the soil community at this organic farm, possibly because organic matter inputs, and crop rotations may have selected for a complex and stable soil biotic community.

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References

Andrassy I 1956 Die Rauminhalts und Gewichtsbestimmung der Fadenwürmer (Nematoden). Acta Zoo. Acad. Sci., Hung. 2, 1–15.

Arines J, Vilarino A and Sainz M 1989 Effect of different inocula of vesicular–arbuscular mycorrhizal fungi on manganese content and concentration in red clover (*Trifolium pretense* L.) plants regenerated from selected cell cultures. Soil Sci. Plant Nutr. 37, 699–705.

Bakhtiar Y, Miller D, Cavagnaro T R and Smith S E 2001 Interactions between two arbuscular mycorrhizal fungi and fungivorous nematodes and control of the nematode with fenamifos. Appl. Soil Ecol. 17, 107–117.

Barker K R 1985 Nematode extraction and bioassays. *In* An Advanced Treatise on Meloidogyne, Vol 2 Methodology. Eds. K R Barker, C C Carter and J N Sasser. pp. 19–35. North Carolina State University Graphics, Raleigh, NC.

Barker S J, Stummer B, Gao L, Dispain I, O'Connor P J and Smith S E 1998 A mutant in *Lycopersicon esculentum* Mill with highly reduced VA mycorrhizal colonization, isolation and preliminary characterisation. Plant J. 15, 791–797.

Bligh E G and Dyer W M 1959 A rapid method of lipid extraction and purification. Can. J. Biochem. Phys. 35, 911–917.

Bossio D A and Scow K M 1998 Impacts of carbon and flooding on soil microbial communities, phospholipid fatty acid profiles and substrate utilization patterns. Microbial Ecol. 35, 265–278.

Bossio D A, Scow K M, Gunapala N and Graham K J 1998 Determinants of soil microbial communities, effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. Microbial Ecol. 36, 1–12.

Burleigh S H, Cavagnaro T R and Jakobsen I 2002 Functional diversity of arbuscular mycorrhizas extends to expression of plant genes involved in P nutrition. J. Exp. Bot. 53, 1–9.

Carpenter-Boggs L, Kennedy A C and Reganold J P 2000 Organic and biodynamic management, effects on soil biology. Soil Sci. Soc. Am. J. 64, 1651–1659.

Cavagnaro T R, Smith F A, Hay G, Carne-Cavagnaro V L and Smith S E 2004 Inoculum type does not affect overall resistance of an arbuscular mycorrhiza-defective tomato mutant to colonisation but inoculation does change competitive interactions with wild-type tomato. New Phytol. 161, 485–494.

Cavagnaro T R, Smith F A, Smith S E and Jakobsen I 2005 Functional diversity in arbuscular mycorrhizas, exploitation of soil patches with different phosphate enrichment differs among fungal species. Plant Cell Environ. 28, 642–650.

David-Schwartz R, Badani H, Smadar W, Levy A A, Galili G and Kapulnik Y 2001 Identification of a novel genetically

controlled step in mycorrhizal colonization, plant resistance to infection by fungal spores but not extra-radical hyphae. Plant J. 27, 561–569.

de Deyn G B, Raaijmakers C E, Zoomer H R, Berg M P, de Ruiter P C, Verhoef H A, Bezemer T M and van der Putten W H 2001 Soil invertebrate fauna enhances grassland succession and diversity. Nature 422, 711–713.

Dixon R K, Garrett H E and Cox G S 1989 Boron fertilization, vesicular–arbuscular mycorrhizal colonization and growth of *Citrus jambhiri* Lush. J. Plant Nutr. 12, 687–700.

Djajakirana G, Joergensen R G and Meyer B 1996 Ergosterol and microbial biomass relationship in soil. Biol. Fert. Soils 22, 299–304.

Drinkwater L E, Letourneau D K, Workneh F, Van Bruggen A H C and Shennan C 1995 Fundamental differences between conventional and organic tomato agroecosystems in California. Ecol. Appl. 5, 1098–1112.

Duc G, Trouvelot A, Gianinazzi-Pearson V and Gianinazzi S 1989 First report of non-mycorrhizal plant mutants (Myc-) obtained in pea (*Pisum sativum* L.) and fababean (*Vicia Faba* L.). Plant Sci. 60, 215–222.

Elliott E T 1986 Aggregate structure and carbon, nitrogen, and phosphorus in native and cultivated soils. Soil Sci. Soc. Am. J. 50, 627–633.

Facelli E and Facelli J M 2002 Soil phosphorus heterogeneity and mycorrhizal symbiosis regulate plant intra-specific competition and size distribution. Oecologia. 133, 54–61.

Ferris H and Matute M 2003 Structural and functional succession in the nematode fauna of a soil food web. Appl. Soil Ecol. 23, 93–110.

Ferris H, Bongers T and de Goede R G M 2001 Nematode faunal profiles of soil ecosystems. J. Nematol. 33, 257.

Fitter A H and Sanders I R 1992 Mycorrhizal functioning, interactions with the soil fauna. *In* Mycorrhizal Functioning. Eds. M F Allen and E Allen. pp. 333–354. Chapman & Hall, NY.

Forster J C 1995 Soil nitrogen. *In* Methods in Applied Soil Microbiology and Biochemistry. Eds. K Alef and P Nannipiero. pp. 79–87. Academic Press, San Diego, CA.

Frey B and Schuepp H 1993 Acquisition of nitrogen by external hyphae of arbuscular mycorrhizal fungi associated with *Zea mays* L. New Phytol. 124, 221–230.

Gange A C 2000 Arbuscular mycorrhizal fungi, collembola and plant growth. Trends Ecol. Evol. 15, 369–372.

Gao L-L, Delp G and Smith S E 2001 Colonization patterns in a mycorrhiza-defective mutant tomato vary with different arbuscular-mycorrhizal fungi. New Phytol. 151, 477–491.

Gianinazzi-Pearson V, Gianinazzi S, Guillemin JP, Trouvelot A and Duc G 1991 Genetic and cellular analysis of resistance to vesicular aubuscular (VA) mycorrhizal fungi in pea mutants. *In* Advances in Molecular Genetics of Plant–Microbe Interactions. Eds. H Hennecke and D P S Verma. pp. 336–342. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Giovannetti M and Mosse B 1980 An evaluation of techniques for measuring vesicular–arbuscular mycorrhizal infection in roots. New Phytol. 84, 489–500.

Graham J H, Leonard R T and Menge J A 1981 Membrane-mediated decrease of root-exudation responsible for phosphorus inhibition of vesicular–arbuscular mycorrhiza formation. Plant Physiol. 6, 548–552.

Green C T and Scow K M 2000 Analysis of phospholipid fatty acids (PLFA) to characterize microbial communities in aquifers. Hydrogeol. J. 8, 126–141.

Guckert J B, Hood M A and White D C 1986 Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*, increase in the *trans/cis* ratio and proportions of cyclopropyl fatty acids. Appl. Env. Microb. 52, 794–801.

Harrison M J 1999 Molecular and cellular aspects of the arbuscular mycorrhizal symbiosis. An. Rev. Plant Physiol. 50, 361–389.

Hart M M and Reader R J 2003a Does percent root length colonization and soil hyphal length reflect the extent of colonization for all AMF? Mycorrhiza 12, 297–301.

Hart M M and Reader R J 2003b Ergosterol and mycorrhizal fungi, the way forward. New Phytol. 159, 536–537.

Heipieper H J, Diffenbach R and Kewwloh H 1992 Conversion of *cis* unsaturated fatty acids to *trans*, a possible mechanism for the protection on phenol-degrading *Pseudomonas putida* P8 from substrate toxicity. Appl. Env. Microb. 58, 1847–1852.

Hetrick B A D 1991 Mycorrhizas and root architecture. Experientia 47, 355–362.

Hole D G, Perkins A J, Wilson J D, Alexander I H, Grice P V and Evans A D 2005 Does organic farming benefit biodiversity?. Biol. Conserv. 122, 113–130.

Hurst C J, Knudsen G R, McInerney M J, Stetzenbach L D and Walter M V (Eds.) 1996 Manual of Environmental Microbiology. American Society for Microbiology, Washington, DC.

Jackson R B, Manwaring J H and Caldwell M M 1990 Rapid physiological adjustment of roots to localized soil enrichment. Nature 344, 58–60.

Johansen A, Jakobsen I and Jensen E S 1993 External hyphae of vesicular–arbuscular mycorrhizal fungi associated with *Trifolium subterraneum*. 3. Hyphal transport of ^{32}P and ^{15}N. New Phytol. 124, 61–68.

Johansson J F, Paul L R and Finlay R D 2004 Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microb. Ecol. 48, 1–13.

Johnson N C, Graham J H and Smith F A 1997 Functioning of mycorrhizal associations along the mutualism–parasitism continuum. New Phytol. 135, 575–586.

Koide R T 1991 Density-dependent response to mycorrhizal infection in *Abutilon theophrasti* Medic. Oecologia 85, 389–395.

Kothari S K, Marschner H and Romheld V 1991 Effect of vesicular–arbuscular mycorrhizal fungus and rhizosphere microorganisms on manganese reduction in the rhizosphere and manganese concentrations in maize (*Zea mays* L). New Phytol. 117, 649–655.

Lambers H, Chapin F S III and Pons T L 1998 Plant Physiology Ecology. Springer-Verlag, NY, USA.

Lundquist E J, Scow K M, Jackson L E, Uesugi S L and Johnson C R 1999 Rapid response of soil microbial communities from conventional, low input, and organic farming systems to a wet/dry cycle. Soil Biol. Biochem. 31, 1661–1675.

Mäder P, Fliessbach A, Dubois D, Gunst L, Fried P and Niggli U 2002 Soil fertility and biodiversity in organic farming. Science 296, 1694–1697.

Marschner H. 1995 Mineral Nutrition of Higher Plants. 2nd Ed. Academic Press Ltd, San Diego, CA.

Marschner H and Dell B 1994 Nutrient uptake in mycorrhizal symbiosis. Plant and Soil 159, 89–102.

Marschner P and Baumann B 2003 Changes in bacterial community structure induced by mycorrhizal colonisation in split-root maize. Plant and Soil 251, 279–289.

Marschner P, Crowley D E and Lieberei R 2001 Arbuscular mycorrhizal infection changes the bacterial 16S rDNA community composition in the rhizosphere of maize. Mycorrhiza 11, 297–302.

Menge J A 1983 Utilization of vesicular–arbuscular mycorrhizal fungi in agriculture. Can. J. Bot. 61, 1015–1024.

Miller R M and Jastrow J D 2000 Mycorrhiza fungi influence soil structure. *In* Arbuscular Mycorrhizas, Physiology and Function. Eds. Y Kapulnik and D D Douds. pp. 3–18. Kluwer Academic Publishers, Dordrecht, The Netherlands.

Miller R L and Jackson L E 1997 Survey of vesicular–arbuscular mycorrhizae in lettuce production in relation to management and soil factors. J. Agr. Sci. 130, 173–182.

Miranda K M, Espey M G and Wink D A 2001 A rapid, simple spectrophotometric method for simultaneous determination of nitrate and nitrite. Nitrc. oxide 5, 62–71.

Morgan J A 1986 The effects of nitrogen nutrition on the water relations and gas exchange characteristics of wheat (*Triticum aestivum*). Plant Physiol. 80, 52–58.

Nichols P D, Smith G A, Antworth C P, Hanson R S and White D C 1985 Phospholipid and lipopolysaccharide normal and hydroxyl fatty acids as potential signatures for methoan-oxidizing bacteria. FEMS Microb. Ecol. 31, 327–335.

Olsson P A, Bååth E, Jakobsen I and Söderström B 1995 The use of phospholipid and neutral lipid fatty acids to estimate biomass of arbuscular mycorrhizal fungi in soil. Mycol. Res. 99, 623–629.

Olsson P A, Bååth E, Jakobsen I and Söderström B 1996 Soil bacteria respond to presence of roots but not to arbuscular mycorrhizal mycelium. Soil Biol. Biochem. 28, 463–470.

Olsson P A, Jakobsen I and Wallander H 2002 Foraging and resource allocation strategies of mycorrhizal fungi in a patchy environment. Ecol. Stud. 157, 93–115.

Olsson P A, Larsson L, Bago B, Wallander H and van Aarle I M 2003 Ergosterol and fatty acids for biomass estimation of mycorrhizal fungi. New Phytol. 159, 7–10.

Paulitz T C and Linderman R G 1989 Interactions between fluorescent Pseudomonads and VA mycorrhizal fungi. New Phytol. 113, 37–45.

Perrin R 1990 Interactions between mycorrhizae and diseases caused by soil-borne fungi. Soil Use Manage. 6, 189–195.

Phillips J M and Hayman D S 1970 Improved procedures for clearing roots and staining parasitic and vesicular–arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycol. Soc. 55, 158–161.

Poulsen K H, Nagy R, Gao L-L, Smith S E, Bucher M, Smith F A and Jakobsen I 2005 Physiological and molecular evidence for Pi uptake via the symbiotic pathway in a reduced mycorrhizal colonization mutant in tomato associated with a compatible fungus. New Phytol. 168, 445–454.

Robertson G P, Klingensmith K M, Klug M J, Paul E A, Crum J R and Ellis B G 1997 Soil resources, microbial activity, and primary production across an agricultural ecosystem. Ecol. Appl. 7, 158–170.

Ryan M H and Angus J F 2003 Arbuscular mycorrhizae in wheat and field pea crops on a low P soil: increased Zn-uptake but no increase in P-uptake or yield. Plant and Soil 250, 225–239.

Sah R N and Miller R O 1992 Spontaneous reaction for acid dissolution of biological tissues in closed vessels. Anal. Chem. 64, 230–233.

Shangguan Z P, Shao M A and Dyckmans J 2000 Nitrogen nutrition and water stress effects on leaf photosynthetic gas exchange and water use efficiency in winter wheat. Env. Exp. Bot. 44, 141–149.

Smith S E and Read D J 1997 Mycorrhizal Symbiosis. 2nd Ed. Academic Press Ltd, Cambridge, UK.

St John T V, Coleman D C and Reid C P P 1983 Growth and spatial distribution of nutrient absorbing organs, selective exploitation of soil heterogeneity. Plant and Soil 71, 487–493.

Steenwerth K L, Jackson L E, Calderón F J and Stromberg M R 2002 Soil microbial community composition and land use history in cultivated and grassland ecosystems of coastal California. Soil Biol. Biochem. 34, 1599–1611.

Sukarno N, Smith S E and Scott E S 1993 The effect of fungicides on the vesicular–arbuscular mycorrhizal symbiosis. I. The effects on vesicular–arbuscular mycorrhizal fungi and plant growth. New Phytol. 123, 139–147.

Tenuta M and Ferris H 2004 Sensitivity of nematode life-history groups to ions and osmotic tensions of nitrogenous solutions. J. Nematol. 36, 85–94.

ter Braak C J F 1987 Ordination. *In* Data Analysis in Community and Landscape Ecology. Ed. R H Jongman, et al. pp. 91–169. Center for Agricultural Publishing and Documentation, Wageningen, The Netherlands.

Tibbett M 2000 Roots, foraging and the exploitation of soil nutrient patches, the roles of mycorrhizal symbiosis. Funct. Ecol. 14, 397–399.

Tisdall J M and Oades J M 1979 Stabilization of soil aggregates by the root systems of ryegrass. Aust. J. Soil Res. 17, 429–441.

Tisdall J M 1991 Fungal hyphae and structural stability of soil. Aust. J. Soil Res. 29, 729–743.

Vance E D, Brookes P D and Jenkinson D S 1987 An extraction method to estimate soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.

Villegas J and Fortin J A 2001 Phosphorus solubilization and pH changes as a result of the interactions between soil bacteria and arbuscular mycorrhizal fungi on a medium containing NH_4^+ as nitrogen source. Can. J. Bot. 79, 865–870.

Wamberg C, Christensen S, Jakobsen I, Muller A K and Sorensen S J 2003 The mycorrhizal fungus (*Glomus intraradices*) affects microbial activity in the rhizosphere of pea plants (*Pisum sativum*). Soil Biol. Biochem. 35, 1349–1357.

Welch R M and Graham R D 2004 Breeding for micronutrients in staple food crops from a human nutrition perspective. J. Exp. Bot. 55, 353–364.

Zar J H 1999 Biostatistical Analysis. Prentice Hall, New Jersey, USA.

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