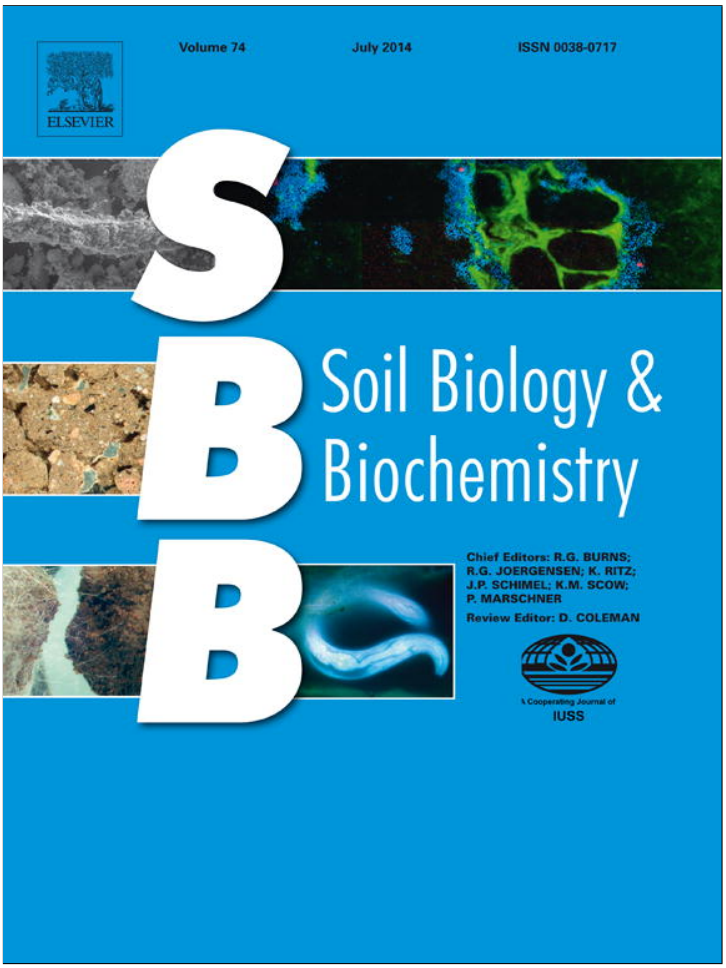

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Arbuscular mycorrhizal effects on plant water relations and soil greenhouse gas emissions under changing moisture regimes

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ABSTRACT

Increased nutrient and/or water uptake by AM symbiosis may affect soil biochemical properties and greenhouse gas (GHG) emissions. A greenhouse experiment was carried out to compare mycorrhizal tomato (76R MYC) and its non-mycorrhizal mutant (*rmc*) on the CO_2 and N_2O emissions from an organically-managed soil. Plants were grown for 10 weeks in pots with compost amended soil and subjected to two consecutive dry down cycles to simulate changing moisture regimes in the field. Dry downs were applied gradually through controlled watering treatments. The effects of AM and soil moisture in GHG emissions were assessed in root in-growth PVC cylinders installed in the pots. Gas samples were taken from the cylinders using static chambers 4 h after each watering event. Photosynthetic rates and stomatal conductance of the plants were assessed after watering using a field portable open flow infra-red gas analyzer. Soil moisture was monitored throughout the experiment. Plant biomass and total shoot N, P and K as well as soil content of DON, DOC, NH_4^+–N, NO_3^-–N and microbial biomass C, were assessed at harvest. For the same shoot growth and nutrient content, *rmc* plants allocated more resources to root biomass than mycorrhizal plants. AM symbiosis improved the capacity of the plants to adapt to changing soil moisture, increasing photosynthetic rates and stomatal conductance at high soil moisture but decreasing them when soil moisture was lower. In addition AM symbiosis helped to regulate N_2O emissions at high soil moisture. Control over N_2O emissions by AM plants seemed to be driven by a higher use of soil water and not by increased N uptake.

1. Introduction

Interactions between roots and soil microorganisms control nutrient availability and uptake by plants and affect soil greenhouse gas (GHG) emissions (carbon dioxide (CO_2) and nitrous oxide (N_2O)) (Jackson et al., 2008; Philippot et al., 2008; Frank and Groffman, 2009). Arbuscular mycorrhizal (AM) fungi constitute one of the most widespread root-microorganism symbiotic associations (Smith and Smith, 2011). This symbiosis increases the uptake of soil nutrients in exchange for photoassimilated carbon compounds (Kiers et al., 2011; Fellbaum et al., 2012). In addition to their well-known role in P nutrition, AM symbiosis mediate the uptake of several forms of soil N, particularly inorganic N (Mäder, 2008; Ruzicka et al., 2012). Using a wild-type mycorrhizal tomato plant and a closely related reduced mycorrhizal colonization mutant (*rmc*) (Barker et al., 1998), Ruzicka et al. (2012) showed that mycorrhizal roots use soil N differently than *rmc* plants based on the expression of different genes involved in N uptake. In addition, higher N uptake is generally observed in the mycorrhizal genotype (Cavagnaro et al., 2006, 2012). By enhancing N uptake and assimilation, AM symbiosis reduces the risk of N loss through nitrate leaching (Asghari and Cavagnaro, 2012), and could also reduce the loss of N from the ecosystem as N_2O. Yet, few studies have been conducted on the role of AM symbiosis on N_2O emissions (Veresoglou et al., 2012). Cavagnaro et al. (2012) observed that field-grown plants of the mycorrhizal tomato genotype had higher N uptake but no effect on soil N_2O emissions. This same study, reported higher CO_2 emissions in the mycorrhizal plants than in the non mycorrhizal mutants. It has been previously suggested that the AM symbiosis can influence soil CO_2 emissions either due to direct respiration of the fungi or due to indirect impacts on heterotrophic microorganisms (Johnson et al., 2002; Langley and Hungate, 2003; Zhu and Miller, 2003; Cavagnaro et al., 2008).

The AM symbiosis may also influence soil biogeochemical processes and GHG emissions through the change in soil physical properties such as soil water holding capacity (Augé, 2004;

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Cavagnaro et al., 2006). Soil GHG emissions are largely conditioned by the prevailing soil moisture. Soil water content has large influences in soil microbial communities altering mineralization, gas diffusivity, oxygen availability, nitrification and denitrification processes (Blagodatsky and Smith, 2012). Previous experiments in agricultural soils in California observed that CO_2 emissions peaked at 60% water-filled pore space (WFPS) whereas N_2O emissions were larger at higher soil moisture (>60% WFPS) (Burger et al., 2005). Besides the indirect effects on soil water retention, a large body of scientific evidence shows that AM symbiosis modifies plant water relations making them more resistant to water stress as compared to non-mycorrhizal plants (see review by Augé, 2001). When soil moisture decreases, stomatal conductance generally remains unaffected for longer in mycorrhizal than non-mycorrhizal plants (Duan et al., 1996). Similarly, mycorrhizal plants frequently exhibit higher photosynthetic rates at lower soil moisture conditions, showing a higher tolerance to drought and intrinsic water use efficiency (Augé, 2001; Ruiz-Lozano et al., 2012). The larger plant size typical of mycorrhizal plants may increase deeper exploration of soil water and nutrients by hyphae and thus result in a higher photosynthetic rate (Augé, 2001; Birhane et al., 2012). Nevertheless, differences in water relations between mycorrhizal and non-mycorrhizal plants are also observed between plants of similar size and nutrient content (Kothari et al., 1990). If mycorrhizal plants maintain higher stomatal conductance as soil dries, their greater water absorption could result in lower soil moisture compared to non-mycorrhizal plants, thus potentially altering soil biogeochemical cycles and GHG emissions (Augé, 2001, 2004).

The use of fungicides and tillage by intensive agriculture has shown to dramatically reduce the presence of the AM symbiosis (Kjøller and Rosendahl, 2000; Oehl et al., 2004). The role of AM symbiosis on plant and soil GHG emissions might be particularly important in organically-managed systems where the use of fungicides is avoided. Further, in these systems nutrient inputs are usually lower and provided in the form of slow-release organic fertilizers such as composted materials. Thus, the reliance on biological soil interactions for the release and uptake of plant nutrients is higher than in conventional cropping systems (Drinkwater et al., 1995). AM symbiosis of plant roots has the potential to increase water and nutrient use efficiency and thereby improve environmental quality in agroecosystems (Gianinazzi et al., 2010; Jackson et al., 2012). Understanding the role of this and other plant–microbe interactions in plant nutrition and biogeochemical cycles is therefore critical for the development of sustainable agricultural practices based in the intensification of naturally-existing ecological processes. This study examined how the AM symbiosis influenced the nutrition, water relations and physiology of tomato plants, as well as the emissions of CO_2 and N_2O from soil under changing soil moisture regimes and organic fertilization with compost. We hypothesized that mycorrhizal plants would decrease N_2O emissions as compared to plants with reduced mycorrhizal colonization through modulation of plant nutrient and water uptake and its effects on soil microbial and biochemical processes. To test this hypothesis, a controlled greenhouse experiment used the mycorrhizal and *rmc* genotypes described above. This allowed the study of the impacts of the AM symbiosis on plant and soil processes, without sterilization of the soil to establish a non-mycorrhizal control, thereby maintaining intact soil microbial processes in the field. In this experiment the two genotypes were grown in a compost-amended soil from an organic farm, rich in organic N, and were subjected to changes in soil moisture to mimic wet–dry cycles and the patchy moisture distribution typically found under field conditions (Burger et al., 2005).

2. Material and methods

2.1. Plants, soil and compost

The mycorrhizal defective tomato mutant with reduced mycorrhizal colonization (*rmc*) and its wild type progenitor *Solanum lycopersicum* cv. 76R MYC (Barker et al., 1998) were grown in a greenhouse pot study. Seeds were surface-sterilized and germinated in 72-well trays with peat moss and grown for 6 wk prior to transplanting. The soil, a fine-silty, mixed, superactive, calcareous, thermic Typic Endoaquepts (16.2% clay, 57.9% silt, 25.8% sand), was collected from an organically managed farm (Durst Organic Growers, Esparto, Yolo County, California) and subsequently sieved through a 1 cm mesh screen. Soil from this farm has been previously reported to provide mycorrhizal colonization of 15–25%, which is typical of well-colonized tomato (Cavagnaro et al., 2006; Ruzicka et al., 2012). After sieving, soil was mixed with sterilized sand in a 4:1 (soil:sand) ratio to increase soil particle size and facilitate gas diffusivity and watering in the greenhouse pots. Final particle size distribution was 9.8% clay, 34.1% silt, and 56% sand. The potting soil was subsequently amended with compost at a rate of 7.8 T ha^{-1} as recommended for processing tomato production in California (Hartz et al., 2008).

The compost was provided by Greenbelt Carriers (Dixon, Yolo County, California) and produced from a mixture of cow (40%), goat (20%), sheep (20%), and horse manure (15%), with oak shavings (5%). Soil and compost properties are summarized in Table 1. Compost amendment provided 34, 2 and 57 mg kg^{-1} of N, P and K respectively. The compost and potting soil were thoroughly mixed and incorporated into the 12 L pots at a bulk density of 1.1 g cm^{-3}, similar to typical field conditions. The effects of moisture regimes and mycorrhizal colonization on soil N_2O and CO_2 emissions from pots were assessed in root in-growth cylinders installed in the pots (Fig. 1). Cylinders were made from PVC pipe 10 cm in depth and 13.5 cm in diameter. Cylinders had 8 evenly spaced holes (3.5 cm in diameter) on the side of the cylinder to facilitate root growth. The bottom of the cylinder and the side holes were covered with plastic 1 mm mesh. Cylinders were filled with the same potting soil/compost mix as the rest of the pot. During pot set up, cylinders were buried 8.5 cm from the soil surface and 1.5 cm was above the surface.

Pots were watered with distilled water to field capacity which was 22% gravimetric soil moisture, prior to transplanting. Briefly, pots were gently watered until excess water started to drip through the bottom; pot weights were recorded after the excess water drained. Subsequently, one 6-wk old tomato seedling (76R MYC or *rmc*) was transplanted in each pot on 16 August, 2011. Seedlings were transplanted at least 5 cm away from the cylinders. There were 40 pots in total with 20 pots per genotype.

2.2. Moisture regimes

The pots were watered daily to 20% gravimetric soil moisture by replenishing water loss from a pre-set pot weight. Two moisture

Table 1
Physicochemical properties of the farmland soil (without compost) and the compost used in the experiment.

	Soil	Compost
Bulk density (g cm^{-3})	1.16	Not applicable
Total C (μg g dw^{-1})	$11.9 \cdot 10^3$	$175 \cdot 10^3$
Total N (μg g dw^{-1})	$1.7 \cdot 10^3$	$10.4 \cdot 10^3$
NH_4^+–N (μg g dw^{-1})	8.31	648.2
NO_3^-–N (μg g dw^{-1})	8.08	1.29
Olsen-P (μg g dw^{-1})	35.4	674
K (μg g dw^{-1})	$3 \cdot 10^3$	$18 \cdot 10^3$
$\delta^{15}N$	4.7	11.4

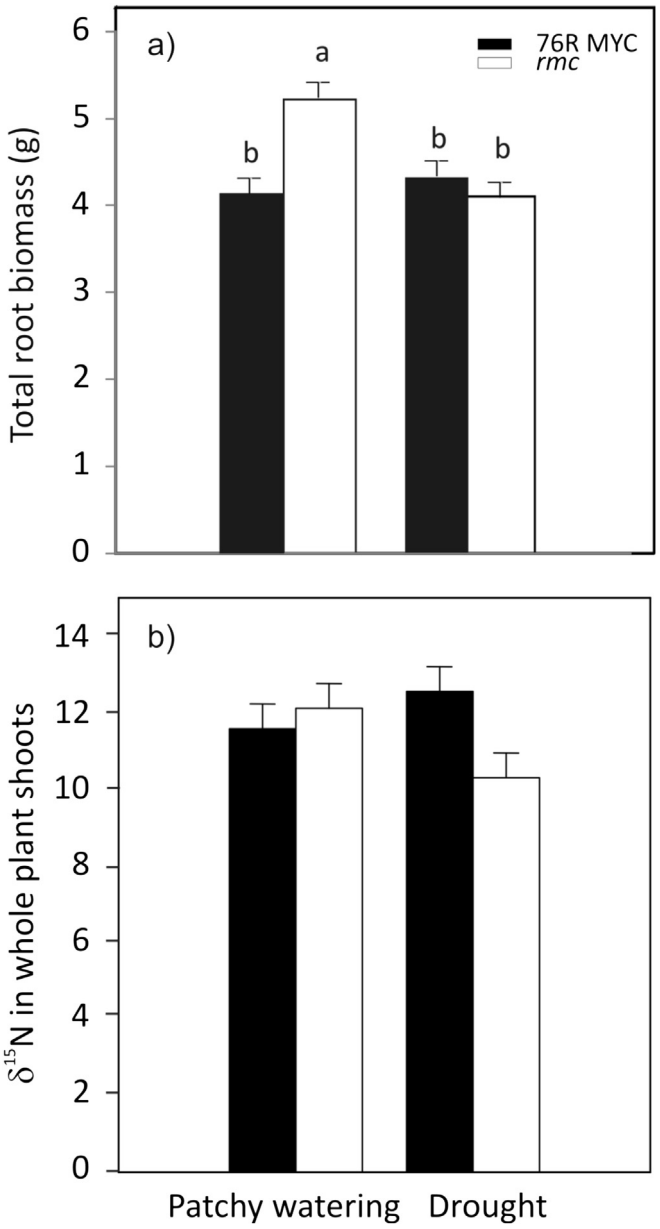

Fig. 1. Total root biomass (a) and $\delta^{15}N$ signature of the shoots (b) of the 76R MYC and *rmc* plants subjected to the drought and patchy watering treatments. Values are means of 10 reps ± standard error. Different letters indicate significant differences between treatments and plant genotypes at $p < 0.05$.

probes (ECH_2O EC-5, Decagon Devices Inc., Washington, USA) were installed in each pot, one in the root in-growth cylinder at 0–5 cm depth, and one at 20 cm below the surface. Probes were used to track soil moisture changes in the pots after watering and at sampling (see below).

Pots were subjected to two controlled dry down cycles with a recovery phase in between. The first dry down cycle was carried out two weeks after transplanting 8-wk old plants, and consisted of a 3% daily reduction in soil moisture over 4-day period (from August 30th to September 2nd), starting at 20% gravimetric moisture and ending at 11%. Soil moisture was monitored and adjusted daily, by weighing the pots and adding water if needed. After first dry down, soil pot moisture was replenished to 20%, and six days later, the 9-wk old plants were subjected to the second dry down cycle over a 9 day period. In this case, two different moisture regimes were applied: (i) a *drought* treatment where moisture of the pots was gradually decreased from 22% to 10% gravimetric moisture over the nine days; and (ii) a *patchy watering* moisture regime, where the pots were subjected to the same decrease in moisture but where the PVC root in-growth cylinders were kept at 22% moisture by watering with a syringe with canulated needles to ensure homogeneous moisture distribution. As in the previous dry down, soil moisture was monitored daily by weighing the pots and moisture was adjusted on alternate days. The amount of water to be added to each in-growth cylinder was based on the loss of total pot weight. The proportional amount of water was calculated to bring the soil volume in the in-growth cylinder to 22% moisture. The changes in soil moisture were monitored right after watering using the 0–5 cm moisture sensors installed in the cylinders, and the 20 cm sensor for the soil in the rest of the pot. Each watering treatment was applied to half of the plants of either genotype, resulting in 10 replicates (pots) for each combination of 'plant genotype' and 'watering treatment' and a total of 40 pots. Pots were arranged in the greenhouse following a randomized block design with 5 blocks, each of them containing 4 replicates per each genotype and 2 replicates per each combination of genotype × treatment.

2.3. Plant growth and N uptake

After the second dry down, shoots were clipped at the soil surface and subsequently dried for one week at 60 °C for dry mass determination. Shoots were ground for the determination of total N and $\delta^{15}N$ as a signature of soil-N source to plants. Stable isotope ratios of N were determined at the UC Davis Stable Isotope Facility (URL: http://stableisotopefacility.ucdavis.edu/), with a continuous flow isotope ratio mass spectrometer (cf-IRMS, Europa Integra; PDZ-Europe Scientific, Sandback, UK). Shoot N content was determined by dry combustion on a Carlo-Erba CHN analyzer (Costech Analytical Technologies, Inc., Valentia, CA). Shoot K and P were determined through atomic emission spectrometry (ICP-AES) in microwave and nitric acid digested samples (http://anlab.ucdavis.edu/). Belowground biomass was determined after wet extraction of the roots from the soil in the pot and from a 500 g soil sample from the in-growth cylinders. Mycorrhizal colonization was determined in a subsample of fine roots extracted from the cylinder in each pot. These roots were cleared with KOH 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 AMF was then determined in root fragments mounted on slides and by counting presence or absence of AM structures (vesicles, arbuscles and hyphae) in the intersection of roots and an eyepiece crosshair arranged perpendicular to the root axis. A total of 50 intersections were assessed per slide. Magnification was ×200 (McGonigle et al., 1990).

2.4. Photosynthetic rates, stomatal conductance and water use efficiency

Leaf gas exchange measurements were conducted during the second dry down, one day after each watering event. Five replicates were chosen for each combination of plant genotype × watering treatment (20 plants in total). A mature, fully expanded leaflet was measured in each plant with a field portable open flow infrared gas analyzer (IRGA) (Model 6400, LI-COR Inc., Nebraska, USA). Measurements were taken between 1000 and 1300 h with a 6 cm^2 chamber, with the CO_2 reference set at 400 μmol m^{-2} s^{-1}, and with saturating light using a LED source (PAR in: 2000 μmol m^{-2} s^{-1}). Water use efficiency (WUE_i) was calculated as the ratio between photosynthetic rate and stomatal conductance for each leaflet.

2.5. Soil analyses

One soil sample (approx 200 g) was taken from each pot and cylinder at harvest. Inorganic N (NH_4^+–N and NO_3^-–N) was determined colorimetrically from the supernatant of 2 M KCl extracted soil samples (Forster, 1995; Miranda et al., 2001). For the analysis of dissolved organic C and N (DOC and DON respectively), soil samples were extracted with 0.5 M K_2SO_4. Subsequently, DOC was analyzed on a Dohrmann Phoenix 8000 UV-persulfate oxidation analyzer (Tekmar-Dohrmann, Cincinnati, OH). For the analysis of DON, K_2SO_4 extracts were persulfate digested to promote oxidation of N forms into NO_3^-–N (Vance et al., 1987; Cabrera and Beare, 1993); DON was calculated as NO_3^-–N in the digested extracts minus NO_3^-–N in the undigested K_2SO_4 extracts determined colorimetrically (Miranda et al., 2001).

For the analysis of microbial biomass C, a soil subsample (25 g fresh weight) was subjected to fumigation with chloroform for 24 h and subsequently extracted with 0.5 M K_2SO_4. Microbial biomass C was calculated as the DOC in the fumigated minus the non-fumigated soil samples (Vance et al., 1987). Available K was determined through ICP-AES in dried and ground soil samples after extraction with ammonium acetate (Thomas, 1982). Available P was determined colorimetrically in $NaHCO_3$ extracted soil and compost samples (Olsen and Sommers, 1982) (http://anlab.ucdavis.edu/). Dried and finely ground soil and compost samples were sent to the UC Davis Stable Isotope Facility for the determination of the $\delta^{15}N$ isotope ratio (URL: http://stableisotopefacility.ucdavis.edu/). The percentage of water filled pore space (WFPS) was calculated from the water content (w) of the soil based on the readings of the moisture sensors installed in the in-growth cylinders and deeper in the pots. Water filled pore space was calculated as follows:

$$\%\text{WFPS} = (w^*\text{bulk density})/[1 - (\text{bulk density}/2.65)]^*100\%$$

2.6. Soil CO_2 and N_2O gas emissions

Soil gas samples were collected from the pots by using a modified static chamber method (Rolston, 1986; Cavagnaro et al., 2012). Chambers consisted of the PVC in-growth cylinders installed in the pots and a PVC cap of the same diameter. Samples (15 mL) were taken at 0, 20 and 40 min after the chamber was capped and stored in pre-evacuated Exetainers (Labco, UK) until analyzed within 15 days.

Preliminary tests showed that gas emissions peaked about 4 h after watering events and underwent a substantial decrease in the following 24 h. Thus, samples were taken 4 h after watering events, daily in the first dry down and every other day in the second. Moisture and temperature (1 and 7 cm depth) of the soil was recorded after each sampling. Samples were analyzed within one week of collection. The CO_2 concentration of the gas samples was determined using an infrared QUBIT S-151 CO_2 analyzer (Qubit Systems Inc., Canada). Concentrations of N_2O were determined with a gas chromatograph with a ^{63}Ni electron capture detector (HP 6890, Hewlett Packard, Palo Alto, CA, USA). Gas concentrations were tested for linearity within the 0, 20 and 40 min samples (Hutchinson and Mosier, 1981) and values were accepted when linear regression coefficient was at least 80% ($R^2 \geq 0.80$). Non-linear fluxes were revised and problematic sampling times were discarded to ensure linearity. Gas fluxes were subsequently converted to mass of gas per area per time (i.e., mg m^{-2} h^{-1}) using the Ideal Gas Law (Baker et al., 2003).

2.7. Data analysis

The effects of soil moisture and AM colonization of roots on plant growth, soil biochemical properties and GHG emissions were analyzed through linear mixed models. For harvest data (plant biomass, plant nutrient content, and soil analyses), the tomato 'plant genotype' (76R MYC vs. *rmc*) and the 'water treatment' (drought vs. patchy) constituted the main fixed factors, while the 'block' was entered in the model as random factor in order to eliminate the potential variability associated with the spatial distribution of the pots in the greenhouse. Also, since plant biomass was found to be significantly and negatively correlated to shoot $\delta^{15}N$, and it was included as a covariate in the mixed model used to test the effect of genotype and drought treatments on shoot $\delta^{15}N$. For data that were collected throughout the experiment such as CO_2, N_2O, photosynthetic rate and stomatal conductance, repeated measures mixed models were used with 'plant genotype' and 'treatment' as fixed factors, 'block' as random factor, 'pot' as the main subject and 'day' was as a within subjects factor. The WFPS of the pots and cylinders, collected simultaneously with the gas samples, were included as covariates in the mixed model in order to test the influence of the changes in soil moisture on the emissions and leaf gas exchange. Interaction between the covariate 'WFPS' and the main factor 'plant genotype' was tested to assess whether the effects of the covariate on the response variables was different between the plant genotypes. A significant interaction would indicate that the correlation between the covariate and the response variable is affected by the fixed factor (plant genotype), resulting in different regression equations (i.e. different slopes and y-intercepts) for each of the genotypes. In all cases, specific comparisons among levels of the same factor were tested for significance by the LSMEAN statement, at $p < 0.05$. Data was tested for normality by Kolmogorov–Smirnov criteria and transformed when necessary by using natural logarithm (ln) and square root transformations. Data analysis was carried out using SAS (SAS Institute, Cary, NC, USA) and SPSS V 20.0 (IBM Corp., NY, USA) software programs.

3. Results

3.1. Plant growth, nutrition and water relations

In roots, colonization of *rmc* plants was low (7%) and restricted to the epidermis, while 76R MYC plants showed a five times higher colonization rate (35.6%). Total root biomass per plant was higher in *rmc* plants than in mycorrhizal plants under patchy watering (Fig. 1a). Furthermore, root biomass was reduced in *rmc* plants in the drought treatment, while it remained unaffected in 76R MYC plants (genotype × treatment: $p = 0.02$). Root length density in the in-growth cylinders was similar between the two genotypes under patchy watering, and was only reduced by the drought treatment in *rmc* plants (genotype × treatment: $p = 0.03$, Table 2). Thus, root length density and biomass in the cylinders was less affected by water treatments in the mycorrhizal genotype.

Shoot biomass was similar between the two genotypes (genotype: $p = 0.55$, 76R MYC: 39.5 ± 1.5 g plant^{-1}; *rmc*: 39.2 ± 1.5 g plant^{-1}) regardless of the soil moisture treatment (Table 2). Also both plant genotypes had similar N, P and K contents in their aerial biomass at harvest and this was not influenced by the soil moisture regime (Table 2). Differences in the shoot $\delta^{15}N$ signatures between the two plant genotypes in response to the watering treatments were close to significantly different at $p = 0.06$ (Fig. 1b). Overall, tomato plants increased their $\delta^{15}N$ signature over the experiment from 4.6 and 4.2 in the 76R MYC and *rmc* seedlings, respectively, to 11.8 ± 0.46 and 10.9 ± 0.46 at harvest for 76R MYC and *rmc* shoots, respectively, with no significant differences between genotypes. Nevertheless under drought the $\delta^{15}N$ was reduced by 13.6% in *rmc* shoots as compared to 76R MYC (Fig. 1b). The two main sources of N in the potting media, compost and soil,

Table 2
Effects of the experimental factors plant genotype (G), watering treatment (T) and their interaction (G*T) on shoot biomass, root length density and shoot nutrient content of the tomato plants at harvest.

	P-values			Patchy watering		Drought	
	G	T	G*T	76R MYC	*rmc*	76R MYC	*rmc*
Shoot biomass (g plant^{-1})	0.55	0.38	0.15	31.8 ± 1.6	35.1 ± 1.6	32.7 ± 1.5	31.4 ± 1.5
Root biomass in cylinder (mg dry root g^{-1} soil)	0.26	0.22	0.03	0.22 ± 0.02ab	0.25 ± 0.02a	0.24 ± 0.02a	0.17 ± 0.02b
Total plant biomass (g)	0.84	0.42	0.14	38.9 ± 1.9	41.03 ± 1.9	40.05 ± 1.9	37.3 ± 1.9
Shoot N (μg g^{-1})	0.25	0.94	0.17	32,055 ± 1141	31,789 ± 1141	30,620 ± 1196	33,379 ± 1196
Shoot P (μg g^{-1})	0.91	0.26	0.49	3000 ± 0.004	3120 ± 0.02	2990 ± 0.004	2930 ± 0.004
Shoot K (μg g^{-1})	0.43	0.18	0.71	41,300 ± 0.94	42,000 ± 0.18	42,500 ± 0.07	43,800 ± 0.07
Total shoot N (mg plant^{-1})	0.32	0.47	0.69	1026 ± 66	1117 ± 66	1005 ± 63	1045 ± 63

had strongly divergent $\delta^{15}N$ signatures (Table 1) and the final mixture in the potting media was calculated to be 4.85, based on the ratio of the two sources.

Photosynthetic rates decreased in response to lower soil moisture in the pots during the second dry down (Fig. 2). Thus, the supply of localized amounts of water in the patchy watering treatment did not have a significant effect on the photosynthetic rates of the plants, irrespective of mycorrhizal colonization (treatment: $p = 0.14$). However, the pattern of photosynthetic responses to moisture availability differed between *rmc* and 76R MYC plants, as shown by the significant interaction between genotype and WFPS of the pot soil (genotype × $WFPS_{POT}$ $p < 0.01$). Mycorrhizal plants had higher photosynthetic rates than *rmc* plants at higher moisture but lower when the WFPS decreased (Fig. 2a). Similarly, stomatal conductance decreased as pots became dryer irrespective of the water applied in the in-growth cylinders (treatment: $p = 0.22$). Again, the decrease in stomatal conductance was higher in 76R MYC plants than *rmc* plants as they reacted more quickly to the decrease in soil moisture, showing lower conductance than *rmc* plants at low soil WFPS (genotype × $WFPS_{POT}$: $p < 0.01$, Fig. 2b). Water use efficiency increased during the dry down, and this increase was higher in 76R MYC than in *rmc* plants (genotype × $WFPS_{POT}$: $p < 0.01$, Fig. 2c). Thus, colonization by AM fungi conditioned a different ecophysiological response of the tomato plants to the changes in soil moisture.

3.2. Soil parameters

The WFPS in the soil in the pots was, on average, lower in 76R MYC plants than in *rmc* plants based on 9 readings of the sensors at 20 cm depth over the 3-wk period (49.4% ± 0.8 vs. 52% ± 0.8, respectively; $p = 0.04$). But these differences between genotypes actually depended on the sampling date (genotype × date: $p < 0.01$, data not shown), being more evident during the first dry down than towards the end of the experiment. Microbial biomass C in the pots and in the in-growth cylinders at the final harvest was not influenced by the AM symbiosis or by the moisture treatments applied to the plants (Table 3). No differences were found in soil DOC and DON between 76R MYC and *rmc* plants at the final harvest, either in the pots or within the in-growth cylinders, irrespective of the moisture treatment (Table 3). The NH_4^+–N content of the pot soil was very low (<1 μg N g^{-1} soil) in all the treatments, but was slightly higher in soil of the 76R MYC plants under the patchy watering treatment ($p = 0.01$; Table 3). No differences between plant genotypes or watering treatments were observed in soil NH_4^+–N in the in-growth cylinders. No significant differences occurred for NO_3^-–N in the pots or the in-growth cylinders (Table 3).

The N_2O emissions from the in-growth cylinders were higher at the start and decreased towards the end of the experiment. Emissions were significantly and positively correlated with soil WFPS in the cylinders (Fig. 3a, $p = 0.006$). Overall, the highest emissions were reached at approximately 60% WFPS. In addition, the AM symbiosis affected the response of N_2O emissions to WFPS ($WFPS_{CYL}$ × genotype: $p = 0.01$; Fig. 4). The slope of the correlation between N_2O emissions and $WFPS_{CYL}$ was higher in soil of *rmc* plants than 76R MYC plants (0.26 vs. 0.02, respectively), such that changes in N_2O emissions with soil moisture were more pronounced with reduced mycorrhizal colonization. Nitrous oxide emissions from soil of *rmc* plants were higher than 76R MYC plants at high WFPS, but decreased more sharply with decreasing soil WFPS, therefore showing lower emissions at low soil WFPS. Nitrous oxide emissions increased with increasing soil temperatures at 1 (T1) and 7 (T7) cm depths (T1: $p < 0.001$; T7: $p < 0.001$, data not shown).

Emissions of CO_2 from the in-growth cylinders showed an opposite response from N_2O, being strongly and negatively correlated with soil WFPS (Fig. 3b, $p = 0.003$). Emissions were lowest when soil was between 20 and 22% gravimetric moisture, or close to 60% WFPS, but increased when soil was drier. CO_2 emissions over the experiment were not correlated with soil temperature at either depth (T1: $p = 0.90$; T7: $p = 0.17$). The presence of 76R MYC or *rmc* did not have a significant influence on the CO_2 emissions from the cylinders ($p = 0.716$, Fig. 3b).

4. Discussion

Previous studies using the 76R MYC wild tomato genotype and its *rmc* mutant grown in the same organically managed soil have shown that AM symbiosis increases the access of plants to soil N (Cavagnaro et al., 2006, 2007). Here, the same plant model was grown under abundant nutrient supply and controlled greenhouse conditions to obtain similar growth and nutrient uptake in the plants. Symbiosis with AM fungi improved the capacity of the tomato plants to respond to changing soil moisture, increasing stomatal conductance and photosynthetic rates in response to soil moisture availability. As an indirect consequence, plants were able to modify soil moisture and reduce soil N_2O emissions, presumably due to an increase in water uptake.

The influence of AM on plant growth and nutrient uptake has shown to be highest in those environments where nutrients are scarce or less available (Mäder, 2008; Birhane et al., 2012; Ruzicka et al., 2012; Veresoglou et al., 2012). In our study, amendment of the organic soil with compost provided enough nutrients to guarantee similar shoot growth and nutrient concentration of both genotypes. Differences between the genotypes in growth and allocation were limited to root biomass that was significantly larger in the *rmc* mutants under the patchy watering treatment; other growth differences can be observed between other mycorrhizal mutants and their wild types (Rillig et al., 2008). Shoot content of the main macronutrients (N, P and K) in mycorrhizal and non-mycorrhizal plants was similar, and exceeded adequate values for processing

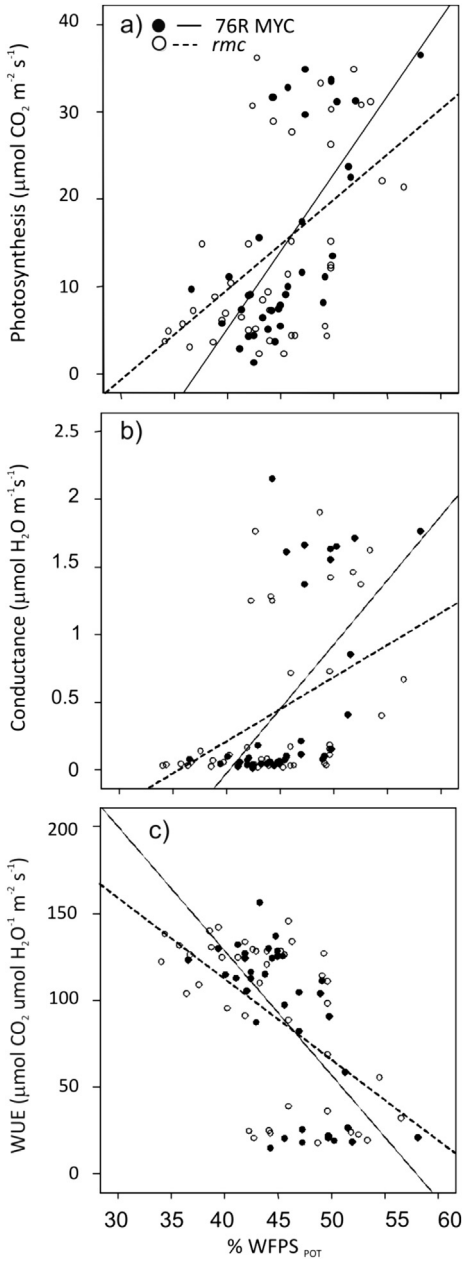

Fig. 2. Effect of the plant genotype on the relationship between photosynthetic rate (genotype × $WFPS_{POT}$ $p < 0.01$; R^2 76R MYC = 0.44; R^2 *rmc* = 0.26) (a), stomatal conductance (genotype × $WFPS_{POT}$ $p < 0.01$; R^2 76R MYC = 0.33; R^2 *rmc* = 0.20) (b), water use efficiency (genotype × $WFPS_{POT}$ $p < 0.01$; R^2 76R MYC = 0.44; R^2 *rmc* = 0.32) (c) and the water filled pore space (WFPS) of the pots over the second dry-down. Full dots and solid line represent the 76R MYC plants while empty dots dotted lines represent *rmc* plants.

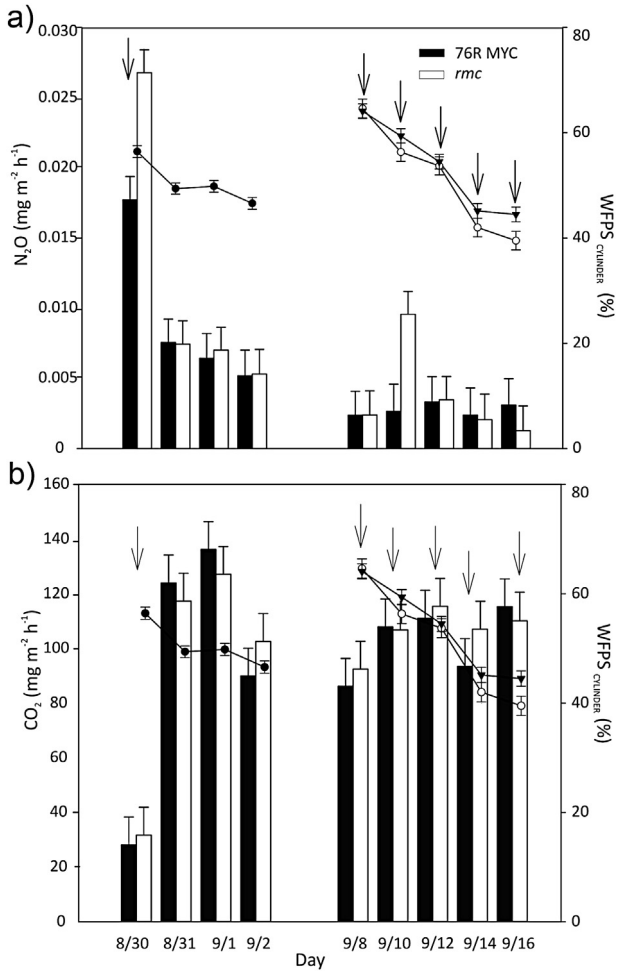

Fig. 3. Nitrous oxide (a) and carbon dioxide (b) emissions from the soils with the 76R MYC and *rmc* plants over the experiment. Line plots on the upper part of the figure indicate the change in WFPS of the pots and rings over the experiment. Vertical arrows indicate watering events.

tomatoes (25,000, 2000 and 25,000 µg g^{-1} of N, P and K respectively; Maynard and Hochmuth, 1997). Besides increasing the uptake and assimilation of N, it has also been postulated that AM symbiosis could determine an increased uptake from certain sources such as organic matter, involving a direct benefit for farmers when organic fertilizers are used (Leigh et al., 2009). Uptake from different sources can be determined through the plant's $\delta^{15}N$ if the signature of the sources is sufficiently different (Evans et al., 1996; Robinson, 2001). The $\delta^{15}N$ of the compost was higher than that of the soil and therefore, a higher signature in the plant would denote a higher use of compost as an N source. Yet, this was not observed, and both plant genotypes had fairly similar shoot $\delta^{15}N$ suggesting that both plant genotypes used the available N sources in a similar way. Only a slight decrease in shoot $\delta^{15}N$ was observed in *rmc* plants under drought.

For the same shoot growth and nutrient uptake, *rmc* plants allocated more resources to root biomass than mycorrhizal plants. Thus, AM colonization of roots allowed a decrease in the resources allocated to root biomass in 76R MYC plants as compared to *rmc* plants, without compromising shoot biomass. Higher root biomass in *rmc* plants was also previously observed by Cavagnaro et al. (2008) in a greenhouse experiment, at two soil P levels. Resources not allocated to the roots might have been invested instead in the maintenance of the AM symbiosis that presumably provided the absorptive area for nutrient uptake through the hyphal network (Fellbaum et al., 2012).

Mycorrhizal plants increased their photosynthetic rates and stomatal conductance at high soil moisture even when their root biomass was smaller than *rmc* plants. These plants also showed a faster stomatal closure in response to the decrease in soil moisture. This conservative strategy in mycorrhizal plants probably avoided the decrease in root biomass and shoot $\delta^{15}N$ observed in *rmc* plants. Therefore changes in shoot $\delta^{15}N$ probably reflected changes in plant growth under stress conditions, as it was observed by Wheeler and Tilak (2000) for a mycorrhizal tropical tree. Many studies have

Table 3
Impacts of the experimental factors plant genotype (G), watering treatment (T) and their interaction (G*T) on microbial biomass C (MBC), dissolved organic C (DOC), dissolved organic N (DON) and inorganic N (NH_4^+ and NO_3^-) of the pots and root in-growth cylinders installed in the pots.

	P-values			Patchy watering		Drought	
	G	T	G*T	MYC	*rmc*	MYC	*rmc*
Soil in the main part of the pot							
MBC (μg C g dw^{-1})	0.36	0.54	0.60	399 ± 18	392 ± 18	397 ± 17	372 ± 17
DOC (μg C g dw^{-1})	0.53	0.05	0.16	71.1 ± 2.7	69.0 ± 2.7	61.8 ± 2.6	67.42 ± 2.6
DON (μg N g dw^{-1})	0.19	0.64	0.42	16.0 ± 6.4	18.9 ± 6.5	13.9 ± 6.2	26.2 ± 6.2
NO_3^-–N (μg N g dw^{-1})	0.36	0.11	0.29	11.6 ± 3.6	11.3 ± 4.6	15.5 ± 5.3	27.8 ± 9.6
NH_4^+–N (μg N g dw^{-1})	0.37	0.58	0.01	0.78 ± 0.07a	0.59 ± 0.07b	0.60 ± 0.08ab	0.69 ± 0.07ab
Soil in the in-growth cylinder							
MBC (μg C g dw^{-1})	0.75	0.63	0.45	315 ± 15	320 ± 16	318 ± 15	306 ± 15
DOC (μg C g dw^{-1})	0.61	0.34	0.59	45.9 ± 2.9	45.9 ± 2.9	47.1 ± 2.7	50.2 ± 2.7
DON (μg N g dw^{-1})	0.57	0.33	0.42	4.6 ± 1.2	4.9 ± 1.2	6.8 ± 1.1	5.1 ± 1.1
NO_3^-–N (μg N g dw^{-1})	0.28	0.24	0.22	0.43 ± 1.3	0.25 ± 1.3	0.36 ± 1.2	3.41 ± 1.3
NH_4^+–N (μg N g dw^{-1})	0.88	0.53	0.32	0.64 ± 0.06	0.58 ± 0.06	0.56 ± 0.06	0.60 ± 0.06

shown that AM symbiosis influences plant water relations by affecting the thresholds for stomatal closure (Augé, 2001; Birhane et al., 2012; Ruiz-Lozano et al., 2012). Generally, colonization of roots is associated with a higher stomatal conductance than non-mycorrhizal plants under drought thereby increasing tolerance to stress (Augé, 2001). Nevertheless faster closing of the stomata in AM plants in response to water stress was also observed (Goicoechea et al., 2004). Growth conditions might play a key role in modulating the plant strategy; in pots, hyphae are not allowed to explore a large volume of soil to scavenge water resources as in the field and this might force a conservative strategy in the plant with a faster stomatal closure when water resources are not available. Similar conditions could be applicable to other environments where the available resources are localized such as in drip-irrigated cropping systems. Under similar growth and nutritional status of the mycorrhizal and *rmc* plants, differences in water relations could

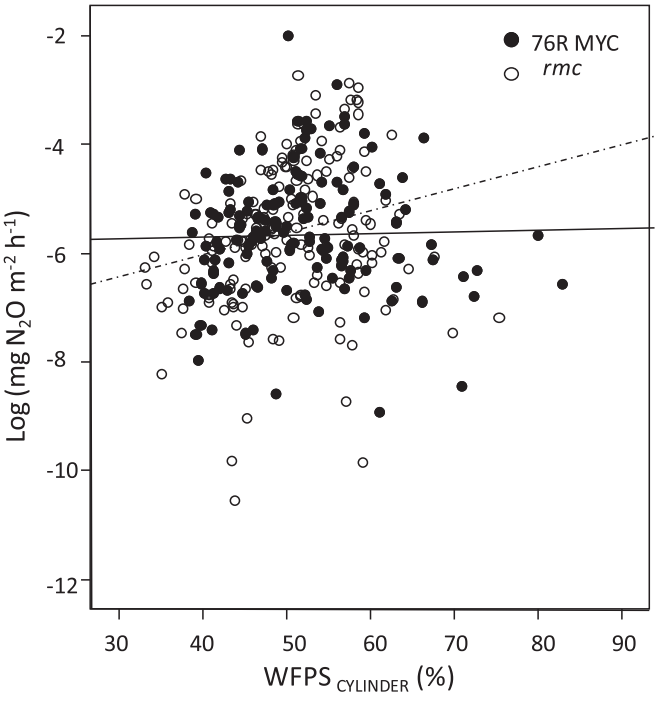

Fig. 4. Effect of the plant genotype on the relationship between the N_2O emissions and the water filled pore space of the cylinders over all the experiment (WFPS × genotype: $p = 0.01$; R^2 76R MYC = 0.004; R^2 *rmc* = 0.05). Full dots and solid line represent the 76R MYC plants while empty dots dotted lines represent *rmc* plants.

be attributed to a higher water uptake through the mycorrhizal network or increased water conductance in the soil through access to micropores (Augé, 2001; Ruiz-Lozano et al., 2012). Regulation of the plant hormones involved in the response to water stress is also possible although not tested here. It has been observed that AM plants regulate better and faster their ABA levels than non-mycorrhizal plants allowing a more adequate balance between leaf transpiration and root water movement during drought and recovery periods (Ruiz-Lozano et al., 2006).

Higher stomatal conductance and use of soil water by AM plants directly influenced soil moisture and N_2O emissions. Yet, no differences were observed between the two plant genotypes in microbial biomass, DOC, DON and inorganic N as it has been previously observed for this organically-managed soil (Cavagnaro et al., 2006, 2007, 2012). Water availability regulates nitrification and denitrification, the main microbial processes leading to the production of N_2O, as well as N availability and gas diffusivity in the soil (Blagodatsky and Smith, 2012). A previous study in an organically managed tomato crop subjected to wet–dry cycles showed higher soil microbial activity and soil N_2O emissions at WFPS higher than 60%, together with short-term pulses of mineralization and available N that further increased the release of N_2O (Burger et al., 2005). Differences in the slope of the correlation between the rate of N_2O emissions and soil WFPS between the mycorrhizal and non-mycorrhizal plants show that the AM symbiosis moderates the magnitude of these pulses, reducing N_2O emissions at high soil moisture. Yet, the low R^2 values obtained for the regression curves, suggest that the ecological significance of this interaction should be further studied with a larger dataset. Reduction of N_2O emissions could be attributed to the higher use of soil water by the AM plants under high soil moisture as compared with *rmc* plants. A priming of the plants through the two experimental dry downs applied could have produced a stronger response of the plants to changes in soil water availability. This stronger response could also induce stronger effects on soil biochemical parameters such as N_2O. Although a priming effect is possible, as plants adapt to wet–dry cycles, this did not have a significant impact on the observed results as N_2O emissions, although different in magnitude, followed a very similar pattern between the first and second dry down. Faster soil drying in AM colonized plants, and its association with higher transpiration rates, have often been noted in comparisons with non-mycorrhizal plants of similar size (Augé, 2001; Marulanda et al., 2003). Effects of increased plant water use were subtle and did not produce a net increase in plant biomass and nutrient uptake, but were enough to be evident at the plant physiological level and in soil N_2O emissions. To the best of our knowledge, only one study investigated the impacts mycorrhizal symbiosis on N_2O emissions (Cavagnaro et al.,

2012). This study reported higher interception of N by 76R MYC plants but failed to detect differences in soil microbial biomass, inorganic N or N_2O emissions (Cavagnaro et al., 2012). Another recent study evaluated the influence of fungal endophytes on soil N_2O emissions (Iqbal et al., 2012) and concluded that the differences in flux rates were due to complex effects on soil microclimate and inorganic N concentrations, yet the governing mechanisms could not be identified.

In contrast to N_2O, AM colonization of roots did not have a significant effect on CO_2 emissions. But for the same amount of CO_2 emitted from the pots, AM colonized plants had significantly lower root biomass than *rmc* plants, especially in the patchy watering treatment. This suggests that in the mycorrhizal plants, a larger portion of the CO_2 emissions might have come from soil heterotrophic respiration. The fact that AM fungi contribute substantially to soil respiration has already been pointed out by Cavagnaro et al. (2008) and Cavagnaro et al. (2012). Between 5 and 20% of the C fixed by mycorrhizal plants is allocated to the fungal mycelium (Pearson and Jakobsen, 1993; Johnson et al., 2002; Bonfante and Genre, 2010; Fellbaum et al., 2012). Yet, a significant portion (3.9–7%) of the C transferred to the AM fungus is released to the atmosphere shortly after fixation (Johnson et al., 2002). Whether the higher CO_2 emissions are due to the respiration of the AM fungus or to the increase in microbial activity due to increased transfer of C to the soil microbial community, remains to be explained.

5. Conclusions

Here, the AM symbiosis improved the capacity of tomato plants to respond to intermittent soil moisture regimes, increasing photosynthesis and stomatal conductance as compared to non-mycorrhizal plants at high soil moisture and more tightly controlling water loss during dry-downs. Soil N_2O emissions were also reduced at high soil moisture with AM colonized plants. Reduction of N_2O emissions seemed to be more related to a higher use of water by the AM plants than to higher use of N in this compost-amended soil. Soil management that enhances the colonization of crop roots by arbuscular mycorrhizae may contribute to a more efficient use of water under changing environmental conditions, reducing also N_2O emissions and therefore the environmental impacts of agricultural practices.

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