Mycorrhizal fungi enhance plant nutrient acquisition and modulate nitrogen loss with variable water regimes

Running header: Mycorrhizae and variable water regimes

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Abstract

Climate change will alter both the amount and pattern of precipitation and soil water availability, which will directly affect plant growth and nutrient acquisition, and potentially, ecosystem functions like nutrient cycling and losses as well. Given their role in facilitating plant nutrient acquisition and water stress resistance, arbuscular mycorrhizal (AM) fungi may modulate the effects of changing water availability on plants and ecosystem functions. The well-characterized mycorrhizal tomato (*Solanum lycopersicum* L.) genotype 76R (referred to as MYC+) and the mutant mycorrhiza-defective tomato genotype *rmc* were grown in microcosms in a glasshouse experiment manipulating both the pattern and amount of water supply in unsterilized field soil. Following 4 weeks of differing water regimes, we tested how AM fungi affected plant productivity and nutrient acquisition, short-term interception of a $^{15}NH_4^+$ pulse, and inorganic nitrogen (N) leaching from microcosms. AM fungi enhanced plant nutrient acquisition with both lower and more variable water availability, for instance increasing plant P uptake more with a pulsed water supply compared to a regular supply and increasing shoot N concentration more when lower water amounts were applied. Although uptake of the short-term $^{15}NH_4^+$ pulse was higher in *rmc* plants, possibly due to higher N demand, AM fungi subtly modulated NO_3^- leaching, decreasing losses by 54% at low and high water levels in the regular water regime, with small absolute amounts of NO_3^- leached (<1 kg N ha^{-1}). Since this study shows that AM fungi will likely be an important moderator of plant and ecosystem responses to adverse effects of more variable precipitation, management strategies that bolster AM fungal communities may in turn create systems that are more resilient to these changes.

Introduction

The two biggest limitations on net primary productivity are nutrient and water availability. Rainfall amounts and patterns are projected to change with climate change (Trenberth et al., 2003), with many regions of the world already experiencing less frequent, but more intense rainfall events (Kirtman et al., 2013). Both the amount and pattern of water supply directly affect plant biomass production and allocation, such as lower productivity and higher root to shoot ratios, with lower and more variable soil water content (Fay et al., 2003; Maestre & Reynolds, 2007; Padilla et al., 2009, 2013; Hagiwara et al., 2010). But water supply also affects biogeochemical processes that mediate nutrient availability to plants and nutrient movement in soil (Porporato et al., 2003; Robertson et al., 2013), which could affect plant nutrient limitation and potential for nutrient losses. For instance, as soil dries, nitrogen (N) and especially phosphorus (P) effectively become less available to plants because mass flow and diffusivity decrease (Moldrup et al., 2001; Suriyagoda et al., 2014), often resulting in lower plant nutrient uptake and higher plant N:P ratios in drought conditions (He & Dijkstra, 2014; Yuan & Chen, 2015). Conversely, more intense wet/dry cycles decrease microbial activity and nutrient transformations during dry periods but cause bursts of activity and available nutrients during rewetting events (Austin et al., 2004; Borken & Matzner, 2009), potentially decoupling plant and microbial processes in time (Dijkstra et al., 2012). If plant growth and nutrient uptake are limited by water availability, causing mobile nutrients to build up in soil, nutrient losses may be higher later when precipitation does occur (Loecke et al., 2017).

Plants have evolved a number of traits to improve resource acquisition in heterogeneous or resource-poor environments, including root architectural modifications, physiological adaptations, and the formation of associations with soil microorganisms, especially arbuscular

mycorrhizal fungi (Lynch, 2007; Lambers *et al.*, 2008; Smith & Read, 2008). Arbuscular mycorrhizas (AM) are formed by most (~80%) terrestrial plants species (Smith and Read 2008), and play a major role in plant nutrient acquisition, especially for less mobile nutrients like P and when nutrient availability is low (Smith et al., 2009; Ruzicka et al., 2011). This includes when soil is dry (Tobar & Barea, 1994; Neumann & George, 2004), and possibly also during pulses of nutrient availability that can occur following rewetting after a dry period, when root competition with soil heterotrophic microbes is high (Borken & Matzner, 2009; Veresoglou et al., 2012). Greater capacity for nutrient uptake in dry soil could in part explain how AM also improve plant performance under drought (for review, see Augé, 2001), especially nutrient interactions with plant water relations and photosynthetic capacity (Evans, 1989; Augé et al., 2015). For instance, in prior work with tomato under field conditions with a 50% deficit irrigation, the AM symbiosis increased tomato yields by 25%, which was associated with greater N and P uptake as well as altered plant water relations but few differences in vegetative biomass (Bowles et al., 2016a). Physiological differences that could explain greater fruit biomass in AM vs. non-AM plants were most apparent immediately following an irrigation after a dry period, when AM plants rapidly increased photosynthesis and stomatal conductance, but non-AM plants did not (Bowles et al., 2016a). This points to the potential importance of AM for mediating plant responses to changes in the *pattern* of water availability, not just the amount, yet most work has only focused only on the amount of water supply without directly manipulating the pattern independently of amounts (Birhane et al., 2012).

In addition to improving plant nutrient acquisition and increasing drought resistance, emerging evidence shows that AM can sometimes reduce nutrient losses following leaching events (Cavagnaro et al., 2015). In some cases, reductions in NO_3^- leaching or N_2O emissions with AM

present have been substantial (Asghari & Cavagnaro, 2012; Bender et al., 2014), but other studies show little effect (van der Heijden, 2010; Cavagnaro et al., 2012; Bender et al., 2015), or a dependency on soil water content, e.g. only decreasing N_2O emissions at higher soil moisture (Lazcano et al., 2014). For reductions in NO_3^- leaching, the pattern of water availability may be a significant factor governing this contingent response, given the potentially enhanced role for AM-mediated N acquisition during low or highly variable soil moisture (Tobar & Barea, 1994; Veresoglou et al., 2012), and for increasing plant drought resistance. If AM can reduce NO_3^- leaching under lower or more variable soil moisture, then the demonstrated adverse effects of altered precipitation patterns on NO_3^- losses in agricultural landscapes (Loecke et al., 2017) could be mitigated with management that supports a robust AM community. For example, the use of cover crops and/or reduced/alternative tillage have been shown to increase colonization of cash crop roots and alter AM fungal community structure (Lekberg & Koide, 2005; Bowles et al., 2016b).

The goal of this study was to determine how AM modulate the response of plant growth, plant nutrient acquisition, and soil nutrient loss to the amount and pattern of water supply. We predicted that the effect of AM on plant growth and nutrition would depend on both the pattern and amount of water availability, and specifically that AM would increase plant N and P uptake more under low and/or more variable water regimes. We also predicted that potential for N leaching would depend on the legacy of water regimes, and that AM fungi would reduce N leaching losses. To investigate mycorrhizal responses, we grew a well-characterized mycorrhiza defective tomato mutant (*rmc*), and its mycorrhizal wild-type progenitor (76R, referred to as MYC+) (Barker et al 1998) in microcosms containing unsterilized field soil. This genotypic approach to establishing experimental treatments avoids impacts of soil sterilization techniques,

which are typically used to establish non-AM comparators, on the wider soil biota and their role in soil nutrient cycling (Watts-Williams and Cavagnaro 2015). The microcosms were subjected to differing watering regimes that independently manipulated the amount and pattern of water availability. We measured plant growth, nutrient uptake, and interception of a ^{15}N pulse, as well as potential for N loss following a simulated leaching event in a glasshouse.

Methods

Overview and experimental design

The experiment was conducted in a glasshouse at the University of Adelaide's Waite Campus (Adelaide, South Australia, Australia) between 14 April and 21 June, 2015. Mycorrhizal and non-mycorrhizal treatments were established using a mycorrhiza defective tomato (*Solanum lycopersicum* L.) mutant with reduced mycorrhizal colonization (named *rmc*) and its mycorrhizal wildtype progenitor (named 76R, referred to here as MYC+) (Barker et al., 1998). The genotypes have similar growth and nutrient uptake when grown in the absence of AM fungi (Cavagnaro et al., 2004), indicating that the mutation affecting the formation of AM in the *rmc* genotype (Larkan et al., 2013) has no pleiotropic effects on other plant processes. Previous work has also shown that the *rmc* locus does not affect interactions with non-AM root colonizing fungi, including *Rhizoctonia solani* AG4 and AG8 (Gao *et al.*, 2006). The amount and pattern of water available to plants was manipulated by establishing three levels of water availability (low, medium, and high, see below), each of which was applied in one of two ways, either daily (regular) or every 3–6 days (pulsed). Thus, there were a total of 12 treatment combinations (2 genotypes × 3 levels of water availability × 2 water regimes), each of which was replicated 5 times for a total of 60 experimental units arranged in a randomized complete block design.

Microcosms, glasshouse conditions, and watering treatments

Microcosms were PVC columns (90 mm diameter × 350 mm height) filled to 300 mm with 2.03 kg of a soil:sand mix (70:30%, w/w) and 107 g of mycorrhizal inoculum to a final bulk density of 1.2 g cm^{-3}. The inoculum was colonized root fragments and soil containing spores and external hyphae of the AM fungus *Rhizophagus irregularis* grown in a 90/10% w/w soil/sand mix. Inoculum was used to bolster the native AM fungi already present in the soil mix to ensure adequate colonization. A PVC cap on the bottom of the column with a 15 mm hole allowed for drainage during the leaching at harvest (see below). No drainage occurred during the experiment. The soil used was a fine sandy loam Urrbrae red-brown earth (Alfisol) collected from the University of Adelaide's Waite Campus Arboretum, South Australia (0–10 cm). The soil was air-dried and sieved to <2 mm prior to use. The soil/sand mix was used to increase particle size, facilitate watering and root extraction, and prevent soil cracking during dry periods. The pH (1:5 soil:water extract) of the mix was 6.2 ± 0.03 and plant-available (Colwell) soil P was 33.5 ± 1.7 μg g^{-1} soil. Soil with adequate levels of available P was used to ensure plant demand for N during the ^{15}N pulse event (see below). Soil NH_4^+-N and NO_3^--N concentrations in the mix, measured colorimetrically on 2M KCl extracts (Foster, 1995; Miranda et al., 2001), were 6.6 ± 0.2 and 35.6 ± 0.5 μg g^{-1} soil, respectively, prior to planting. The gravimetric water content (GWC) of the mix at water holding capacity (WHC) at -10 kPa was 33%, determined using a sintered glass funnel attached to a 1 m water column. The soil/sand mix was packed in the glass funnel to the same bulk density as in the microcosms, saturated with water and allowed to drain for 48 h and then the GWC was determined.

Seeds of 76R and *rmc* were surface sterilized and imbibed prior to planting in trays containing a sterilized coarse sand supplemented with 0.025 g $CaHPO_4$ kg^{-1}. Seedlings were grown for two

weeks in the glasshouse prior to transplanting into microcosms, one plant per pot. Columns were watered to 75% of WHC (by weighing pots) with reverse osmosis (RO) water every second day for 29 days until the different watering treatments began, thereby ensuring that no water leached out of the columns. Plants were grown in a glasshouse with supplemental lighting with a 14.5/9.5 hr light/dark cycle. Mean day time minimum and maximum temperatures were 17.4 °C and 21.2 °C, respectively; mean night time minimum and maximum temperatures were 8.2 °C and 15.6 °C, respectively; mean max and min relative humidity was 49.5% and 81.2%, respectively. At 2 and 4 weeks after transplanting all plants received 20 ml of a modified Long Ashton nutrient solution without P (Cavagnaro et al., 2001), equivalent to 2.2 µg N g^{-1} soil. At 4 weeks after transplanting all plants also received 3.1 mg P as KH_2PO_4 (1.5 µg P g^{-1} soil) and 22.4 mg N as NH_4NO_3 (11 µg N g^{-1} soil) in 100 ml RO water in order to address visual symptoms of nutrient stress prior to imposing the water treatments. As part of routine pest and disease management in the glasshouse, a foliar application of (non-systemic) copper oxychloride was applied to the plants. A plastic barrier was used to prevent the fungicide treatment contacting the soil. This management practice has been found to not adversely impact formation of AM (Cavagnaro, un-published).

The watering treatments were designed to investigate the effects of water availability and heterogeneity, i.e. amount and pattern, following the approaches described in Maestre & Reynolds, (2007), Padilla et al., (2009), and Hagiwara et al., (2010). To produce three levels of water availability (low, medium, and high), the columns were irrigated with different amounts of RO water beginning on 27 May, 2015 (29 days after transplanting) and continuing until plant harvest (25 days later). For each of these levels, the same amount of water was applied either as a single pulse every 3–6 days (“pulsed”) or in smaller quantities daily (“regular”). The total

amount of water applied after starting the differing treatments was 433, 704, and 970 mL in the low, medium, and high amount treatments, corresponding to 6.8, 11.1, and 15.3 cm of water. Soil GWC over the time course is presented in Fig. 1. Gravimetric water content was calculated by weighing pots daily and estimating water content based on the known mass of dry soil, inoculum, and pot for each individual replicate. The mass of the plant was considered negligible compared to the pot and soil (<0.5%).

A pulse of ^{15}N was applied to all pots 7 weeks after transplanting. The pulse was 59.5 mg N pot^{-1} (100 kg N ha^{-1}, or 29.3 µg N g^{-1} soil) as $^{15}NH_4Cl$ at 50 atom percent enrichment (APE). The ^{15}N solution was injected via Sprotte needles into four locations in each pot, 5 mL per location, evenly over a 0–10 cm depth to ensure a uniform application. The ^{15}N was applied immediately prior to watering all pots (i.e. both the regular and pulsed water regimes). At 96 hours after the N pulse, all plants were harvested.

Harvesting and leachate collection

All plants were destructively harvested 8 weeks after transplanting (i.e. 4 days following the ^{15}N pulse). Shoots were cut at the soil surface and a soil core (2 cm diameter, 9 cm deep) was removed for determination of soil moisture, NH_4^+, NO_3^-, and plant-available (Colwell) P prior to the leaching event. The concentration of NH_4^+ and NO_3^- in leachate and 2M KCl extracts of the soil core were determined as above. The hole from the soil core was backfilled with fine sand prior to the leaching event. Columns were then flushed with 700 mL RO water and the leachate collected over 24 hours, at which point all drainage had ceased. The roots were then carefully washed from all of the remaining soil with RO water. Mycorrhizal colonization of a subsample of roots was determined using the gridline intersect method (Giovannetti & Mosse, 1980), following clearing and staining of roots with ink and vinegar (Vierheilig et al., 1998). All

196 remaining plant material was dried at 60 °C, and shoot dry weights and root dry weights
197 determined. Plant tissue was ground to a fine powder. The concentration of P in roots and shoots
198 was determined colorimetrically (Murphy & Riley, 1962) following digestion with nitric acid
199 and hydrogen peroxide (Wheal et al., 2011). All dried plant material was analyzed for total N
200 and $\delta^{15}N$ on a PDZ Europa ANCA-GSL elemental analyzer coupled to a PDZ Europa 20–20
201 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK) at the UC Davis Stable Isotope
202 Facility, USA.

203 *Calculations and statistical analysis*

204 Mycorrhizal responses were calculated using the individual values of shoot and root biomass, N
205 and P concentrations, and N and P content, of MYC+ plants and mean values of these variables
206 of *rmc* plants within each treatment (Watts-Williams *et al.*, 2013; Johnson *et al.*, 2015):

207 $$\%MR = \frac{value\ (MYC+) - mean\ value\ (\text{rmc})}{mean\ value\ (\text{rmc})} \times 100$$

208 Mixed model analysis of variance (ANOVA) was performed using the lme4 and lmerTest
209 packages in R (Bates et al., 2015; Kuznetsova et al., 2016). Genotype, amount, and pattern were
210 treated as fixed effects while block was considered random to account for the randomized
211 complete block design. Degrees of freedom were adjusted based on Kenward and Roger (1997).
212 Transformations were used as needed to meet assumptions of homoscedasticity and normality.

213 **Results**

214 *Water regimes and AM fungal colonization*

215 Following several weeks of plant growth under a uniform watering regime, initiation of the six
216 different water regimes manipulating the amount and pattern of water supply caused substantial

changes in GWC with means of 0.15 ± 0.00, 0.20 ± 0.00, and 0.25 ± 0.00 g g^{-1} in the low, medium, and high water treatments, respectively ($F_{amount,2,44}$=119.8, $p < 0.0001$) over the daily measurements during the whole period (Fig. 1). These soil moisture contents correspond to 45, 60, and 75% of measured field capacity (-10 kPa), and 33, 44, and 55% water-filled pore space, respectively. By contrast, the pattern of water availability did not affect mean GWC ($F_{pattern,1,44}$=0.7, *ns*) but did have a strong influence on the pattern of soil wetting and drying ($F_{pattern\times date,22,1056}$=100.4, $p < 0.0001$); i.e., these microcosms experienced more variable soil moisture. Lower soil moisture in *rmc* microcosms ($F_{genotype,1,44}$=18.9, $p < 0.0001$), especially toward the end of the experiment, likely reflected higher shoot biomass, causing higher transpiration (see below).

Roots from the reduced mycorrhizal tomato genotype (*rmc*) were not colonized by AM fungi (S1 Table). For the wild-type genotype (MYC+), mean colonization was 40%, and this was affected by the watering amount (Table 2), with colonization of 33% at low moisture compared to 47% at high moisture, with the value for the medium water treatment intermediate.

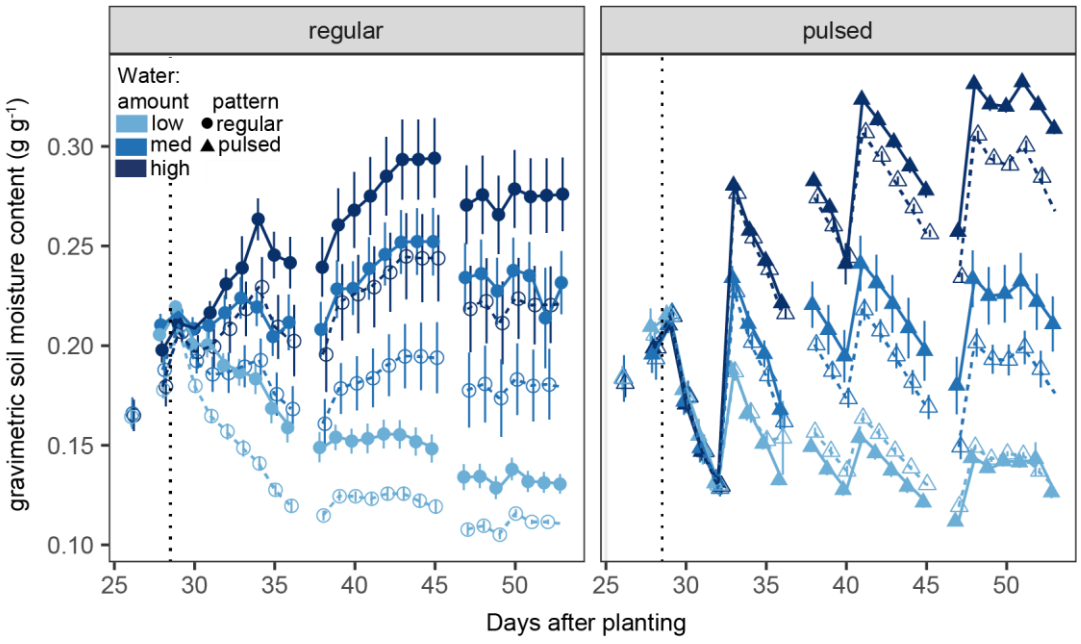

Fig. 1: Gravimetric water content of soil with AM (MYC+) and non-AM (*rmc*) tomato genotypes grown under varying amounts (low, medium and high) and patterns (regular and pulsed) of water supply. Closed symbols are MYC+ and open symbols are *rmc* plants. The vertical dashed line indicates the start of the differing water treatments. For ANOVA results, see text. Soil moisture was not measured on 27, 37, and 46 DAP, so data are not shown.

Plant growth and nutrient uptake

The formation of AM modulated how the amount and pattern of water supply affected plant growth and nutrient uptake, as shown by multiple significant interactions with genotype (Tables 1 and 2, S1 Table). For instance, root biomass was higher in *rmc* than MYC+ in the regular water regime but similar in the pulsed water regime (Tables 1 and 2). To hone in on these effects, differences in the biomass, nutrient concentrations, and nutrient content between AM and non-AM plants, in response to the soil moisture treatments were expressed as mycorrhizal responses (MRs; see Methods). Briefly, MRs were calculated by expressing the difference in a response variable of interest (e.g. biomass) between AM and non-AM plants as a percentage of the non-AM plants. A mycorrhizal benefit existed where the MR was significantly greater than zero (as indicated by 95% CI's), negative when significantly less than zero, or neutral where they were not significantly different from zero.

Table 1. Biomass and N and P concentrations of shoots and roots in mycorrhizal (MYC+) and non-mycorrhizal (*rmc*) tomato genotypes grown under varying patterns and amounts of water. Shown are means ± standard errors. For three-way ANOVA results, see Table 2.

Pattern	Amount	Genotype	Shoot dry weight (g)	Root dry weight (g)	Shoot N conc. (%)	Root N conc. (%)	Shoot P conc. (%)	Root P conc. (%)
Regular	Low	*rmc*	4.79 ± 0.16	2.47 ± 0.07	1.58 ± 0.02	1.70 ± 0.02	0.31 ± 0.01	0.18 ± 0.01
Regular	Low	MYC+	4.38 ± 0.24	2.27 ± 0.15	1.73 ± 0.04	1.74 ± 0.02	0.42 ± 0.01	0.23 ± 0.01
Regular	Medium	*rmc*	5.08 ± 0.33	2.38 ± 0.14	1.71 ± 0.06	1.70 ± 0.03	0.33 ± 0.01	0.18 ± 0.01
Regular	Medium	MYC+	4.43 ± 0.22	2.00 ± 0.14	1.90 ± 0.02	1.86 ± 0.04	0.47 ± 0.02	0.24 ± 0.01
Regular	High	*rmc*	6.42 ± 0.14	2.57 ± 0.11	1.71 ± 0.04	1.77 ± 0.05	0.32 ± 0.01	0.18 ± 0.01
Regular	High	MYC+	5.22 ± 0.15	2.24 ± 0.14	1.77 ± 0.02	1.84 ± 0.02	0.44 ± 0.01	0.23 ± 0.01
Pulsed	Low	*rmc*	4.71 ± 0.15	2.07 ± 0.12	1.63 ± 0.03	1.74 ± 0.03	0.30 ± 0.01	0.16 ± 0.01
Pulsed	Low	MYC+	4.30 ± 0.15	2.38 ± 0.09	1.75 ± 0.02	1.78 ± 0.03	0.36 ± 0.02	0.23 ± 0.01
Pulsed	Medium	*rmc*	5.62 ± 0.26	2.44 ± 0.12	1.63 ± 0.03	1.65 ± 0.03	0.31 ± 0.02	0.16 ± 0.01
Pulsed	Medium	MYC+	4.84 ± 0.15	2.22 ± 0.14	1.81 ± 0.02	1.77 ± 0.04	0.44 ± 0.02	0.23 ± 0.01

Pulsed	High	*rmc*	6.01 ± 0.29	2.29 ± 0.18	1.72 ± 0.01	1.77 ± 0.03	0.32 ± 0.02	0.15 ± 0.01
Pulsed	High	MYC+	5.36 ± 0.13	2.23 ± 0.12	1.82 ± 0.04	1.84 ± 0.04	0.45 ± 0.02	0.22 ± 0.01

Table 2: Summary of linear mixed model ANOVAs for all response variables in mycorrhizal (MYC+) and non-mycorrhizal (*rmc*) tomato genotypes grown under varying patterns and amounts of water. Non-significant effects are not shown. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Variable	Genotype (G)	Amount (A)	Pattern (P)	G×A	G×P	A×P	G×A×P
Colonization (%)	$F_{1,44}=825.7^{***}$	$F_{2,44}=8.5^{***}$		$F_{2,44}=8.5^{***}$			
Shoot dry wt. (g)	$F_{1,44}=36.4^{***}$	$F_{2,44}=15.8^{***}$					
Root dry wt. (g)	$F_{1,44}=3.8^{\#}$				$F_{1,44}=4.6^{*}$		
Root:shoot ratio		$F_{2,44}=3.6^{*}$					
Shoot N conc. (%)	$F_{1,42}=48.6^{***}$	$F_{2,43}=8.7^{***}$				$F_{2,43}=4.6^{*}$	
Root N conc. (%)	$F_{1,44}=21.6^{***}$	$F_{2,44}=5.1^{*}$				$F_{2,44}=3.2^{*}$	
Shoot N content (g)	$F_{1,42}=2.9^{\#}$	$F_{2,43}=22.1^{***}$					
Root N content (g)					$F_{1,44}=4.5^{*}$		
Shoot P conc. (%)	$F_{1,44}=192^{***}$	$F_{2,44}=8.3^{***}$					
Root P conc. (%)	$F_{1,44}=136.5^{***}$		$F_{1,44}=8.2^{**}$				
Shoot P content (g)	$F_{1,44}=55.6^{***}$	$F_{2,44}=21.8^{***}$					
Root P content (g)	$F_{1,44}=38.4^{***}$		$F_{1,44}=4.9^{*}$		$F_{1,44}=7.2^{*}$		
Shoot total ^{15}N (mg)	$F_{1,42}=11.9^{**}$	$F_{2,43}=69^{***}$		$F_{2,42}=5.2^{**}$			
Root total ^{15}N (mg)	$F_{1,44}=4.9^{*}$			$F_{2,44}=4.1^{*}$			
Colwell P (µg P g^{-1} soil)	$F_{1,44}=10.9^{**}$						
Soil NH_4^+ conc. (µg N g^{-1} soil)		$F_{2,44}=4.5^{*}$					
Soil NO_3^- conc (µg N g^{-1} soil)	$F_{1,44}=23.8^{***}$		$F_{1,44}=4.5^{*}$	$F_{2,44}=7.7^{**}$			$F_{2,44}=7.0^{**}$
Leachate volume (mL)	$F_{1,44}=10.1^{**}$	$F_{2,44}=26.0^{***}$				$F_{2,44}=4.7^{*}$	
Leachate, NH_4^+ conc. (µg N mL^{-1})		$F_{2,42}=7.6^{**}$					
Leachate, NO_3^- conc. (µg N mL^{-1})		$F_{2,44}=4.4^{*}$	$F_{1,44}=5.3^{*}$				
Leachate, total NH_4^+ (µg N)							
Leachate, total NO_3^- (µg N)		$F_{2,44}=10.3^{***}$	$F_{1,44}=7.4^{**}$		$F_{1,44}=6.0^{*}$	$F_{2,44}=3.9^{*}$	

Following 3.5 weeks of different watering regimes, AM plants generally had lower biomass than non-AM plants, as shown by MRs of total and shoot biomass that were significantly less than zero (Table 3, Fig. 2), whereas those of root biomass overlapped zero across all water regimes

except plants in the low, pulsed water regime, which showed a significant positive MR for root biomass (Fig. 2). Thus, a growth depression occurred in AM plants in all water regimes but the low, pulsed treatment. The amount and pattern of water supply affected the magnitude of the growth depressions in root and total biomass but not in shoot biomass (Table 3). For instance, root biomass in AM plants was 12.0±6.9% (mean±95% CI) lower than non-AM plants (pooled across each of the water amount treatments) in the regular water regime but similar in the pulsed water regime (Fig. 2).

Table 3: Summary of linear mixed model ANOVAs for all mycorrhizal response variables. Non-significant effects are not shown. MR's were calculated by expressing the difference in a response variable of interest (e.g. biomass) between mycorrhizal and non-mycorrhizal plants as a percentage of the non-mycorrhizal plants. There were no significant amount×pattern interactions. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

Variable	Amount (A)	Pattern (P)
MR (%): Shoot dry wt.		
MR (%): Root dry wt.	$F_{2,20}=4.7^{*}$	$F_{1,20}=9.4^{**}$
MR (%): Total dry wt.	$F_{2,20}=4.1^{*}$	$F_{1,20}=6.0^{*}$
MR (%): Shoot N conc.	$F_{2,20}=7.5^{**}$	
MR (%): Root N conc.	$F_{2,20}=6.7^{**}$	
MR (%): Shoot N content		
MR (%): Root N content		$F_{1,20}=11.3^{**}$
MR (%): Shoot P conc.	$F_{2,20}=3.5^{*}$	
MR (%): Root P conc.		$F_{1,20}=11.7^{**}$
MR (%): Shoot P content		
MR (%): Root P content		$F_{1,20}=25.3^{***}$

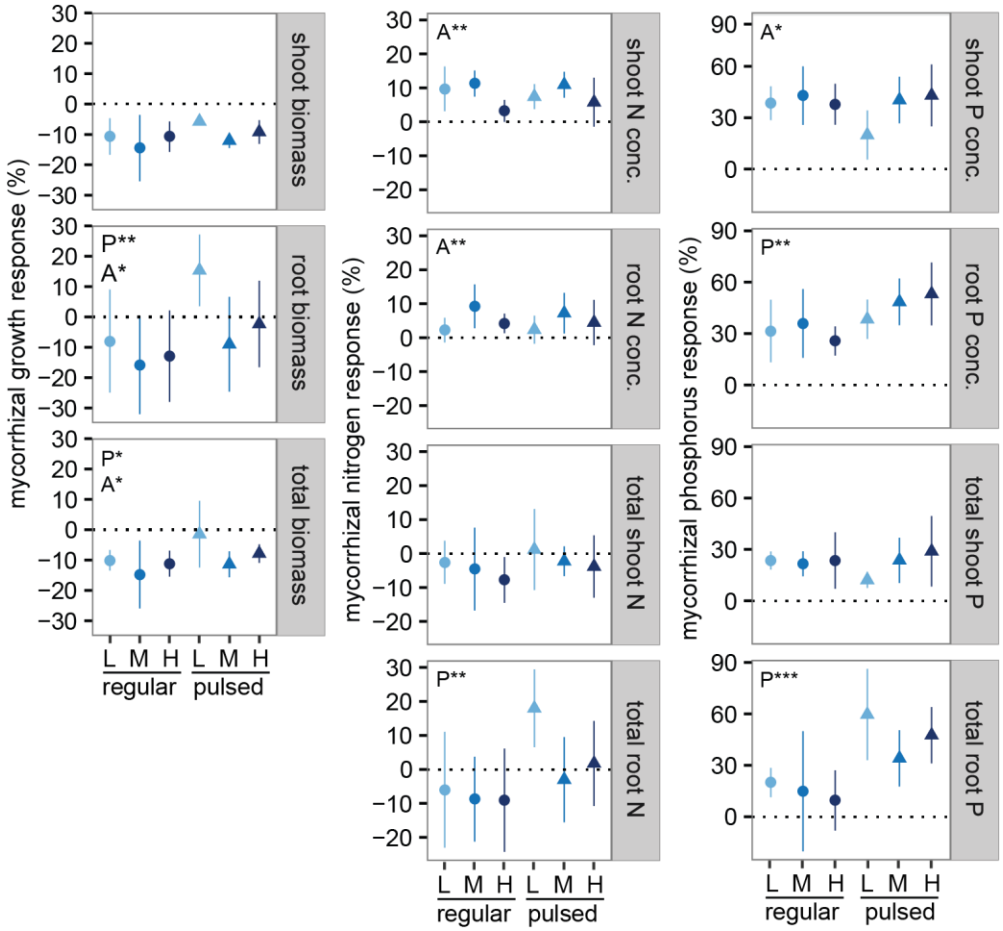

Fig. 2. Mycorrhizal responses (MRs) of tomato shoot and root biomass, N and P concentration, and N and P content. Shown are means ± 95% confidence intervals. MR's were calculated by expressing the difference in a response variable of interest (e.g. biomass) between AM (MYC+) and non-AM (*rmc*) tomato plants as a percentage of the non-AM plants. Tomato genotypes were grown under varying amounts (low, medium and high) and patterns (regular and pulsed) of water supply. A mycorrhizal benefit existed where the MR was significantly greater than zero (as indicated by 95% CI's), negative when significantly less than zero, or neutral where they were not significantly different from zero. On x-axis, L=low, M=medium, and H=high water amounts. Inset in each panel are results of two-way ANOVA (A=amount; P=pattern). *p < 0.05; **p < 0.01; *** p < 0.001.

AM plants typically had higher N and P concentrations and higher P content than non-AM plants, and these responses were dependent on the amount and pattern of water supply (Table 3, Fig. 2). Root P concentration and root P content (i.e. the total amount of P in roots) was 47±7 and 47±10%, respectively, higher in AM plants than non-AM plants in the pulsed water regime vs. only 31±7 and 22±4.2% higher in the regular water regime. Mycorrhizal root systems thus

290 increased plant P acquisition more during the wet/dry cycles of the pulsed regime than the more
291 stable soil moisture conditions of the regular water regime. Higher amounts of water slightly
292 increased the MR of shoot P concentration (Table 2; Fig. 1), increasing from 29±10% in the low
293 treatment to 40±9% in the high treatment.

294 The concentration of N in shoots and roots was generally higher in mycorrhizal plants, but this
295 effect depended on water supply (Fig. 2, Table 3). At low and medium water amounts, the MR of
296 N concentration in shoots was slightly but significantly stronger than at high water amount
297 (8.4±3% at low water vs. 4.5±3% at high water). Shoot and root N content were similar in AM
298 and non-AM genotypes, reflecting higher N concentrations but lower biomass in AM plants. The
299 exception was root N content at low, pulsed water, which was higher in MYC+, mainly
300 reflecting higher root biomass in this group compared to other treatments.

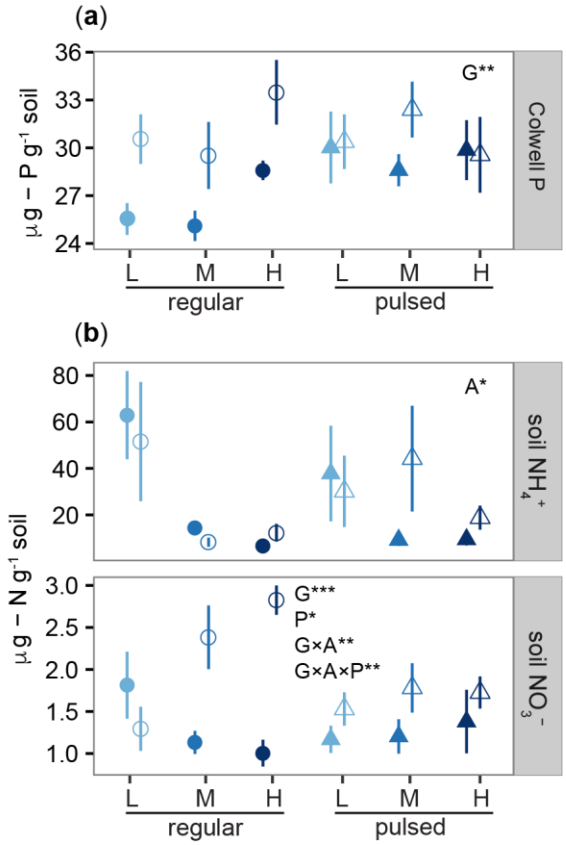

Fig. 3. Colwell P (a), and NH_4^+ and NO_3^- (b) concentrations in surface soil (0–9 cm) of the microcosms at the end of the experiment but prior to leaching, after growth of AM (MYC+) and non-AM (*rmc*) tomato genotypes grown under varying amounts (low, medium and high) and patterns (regular and pulsed) of water supply. Closed symbols are MYC+, and open symbols are *rmc* plants. On x-axis, L=low, M=medium, and H=high water amounts. Inset in each panel are results of three-way ANOVA (A=amount; G=genotype; P=pattern). For ANOVA results, see Table 2. *p < 0.05; **p < 0.01; *** p < 0.001.

At the end of the experiment but prior to the leaching event, residual Colwell P in surface soil was 10.3% higher in microcosms with *rmc* plants than with MYC+ plants, pooled across all other treatments (Fig. 3, Table 2). Surface soil NH_4^+ prior to leaching was high (mean of 25.5 µg NH_4^+-N g^{-1}), likely as a result of the $^{15}NH_4^+$ pulse added to surface soil 4 days prior, and was lower with more water. Surface soil NO_3^- prior to leaching was much lower than soil NH_4^+ (mean of 1.6 µg NO_3^--N g^{-1}) and depended on AM association and the amount and pattern of

water availability. Under the regular water regime, soil NO_3^- in MYC+ microcosms decreased with more water, but soil NO_3^- in *rmc* microcosms had the opposite pattern and increased with more water. In contrast, under the pulsed water regime, soil NO_3^- did not change in MYC+ or *rmc* microcosms across the gradient of water amounts.

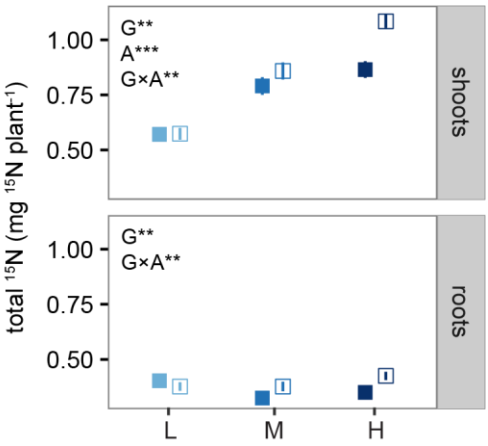

Fig. 4: Recovery of $^{15}NH_4^+$ pulse in shoots and roots of AM (MYC+) and non-AM (*rmc*) tomato genotypes grown under varying amounts (low, medium and high) and patterns (regular and pulsed) of water supply. Results shown here are averaged across the pattern of water supply, which did not have a significant effect on ^{15}N recovery. The $^{15}NH_4^+$ pulse (59.5 mg N pot^{-1}) was applied 4 days prior to harvest. after growth. Closed symbols are MYC+, and open symbols are *rmc* plants. On x-axis, L=low, M=medium, and H=high water amounts. Inset in each panel are results of three-way ANOVA (A=amount; G=genotype; P=pattern). For ANOVA results, see Table 1. *p < 0.05; **p < 0.01; *** p < 0.001.

Recovery of the $^{15}NH_4^+$ pulse by AM vs. non-AM genotypes depended on the amount of water (Fig. 4, Table 2). In shoots, recovery was similar in both genotypes with low and medium watering amounts, but higher in *rmc* than MYC+ at high water. In roots, recovery was slightly higher in MYC+ at low water and higher in *rmc* at medium and high water. Overall, recovery of the $^{15}NH_4^+$ pulse in plants was higher in shoots than roots but in total was low (~4% averaged across all factors), and was not affected by the pattern of watering.

Nitrogen leaching

The volume of leachate collected from the microcosms depended on the water regimes, with the strongest factor being the amount of water applied (Fig. 5; means of 317±7, 370±15, and 436±16 mL in the low, medium, and high water regimes, respectively), reflecting a strong, positive linear relationship between antecedent soil moisture content and leachate volume (R^2=0.73). Leachate volume was also higher in MYC+ microcosms (396±15 vs. 353±12 mL in MYC+ vs. *rmc*, respectively), likely reflecting higher antecedent soil moisture in MYC+ microcosms prior to leaching.

The concentration of NH_4^+ in leachate was similar in low and medium water regimes, but slightly less in high water regimes (Table 2, S1 Table); however, the total amount of NH_4^+-N in leachate did not differ among treatments, with a mean of 9.3 ± 0.5 µg microcosm^{-1}(Fig. 5, Table 2). Both the amount and pattern of water supply affected the concentration of NO_3^--N in leachate (Table 2), with greater NO_3^- concentrations in leachate at low levels of regular watering. The total amount of NO_3^--N leached was higher in the regular water regime compared to the pulsed regime, but only with *rmc* plants. Thus, in the regular water regime, NO_3^- leaching was on average 54% lower in AM plants compared to non-AM plants (274 vs. 125 µg NO_3^--N in *rmc* vs. MYC+, respectively). Also, only in the pulsed water regime, the total amount of NO_3^--N leached was lower with increasing water amounts.

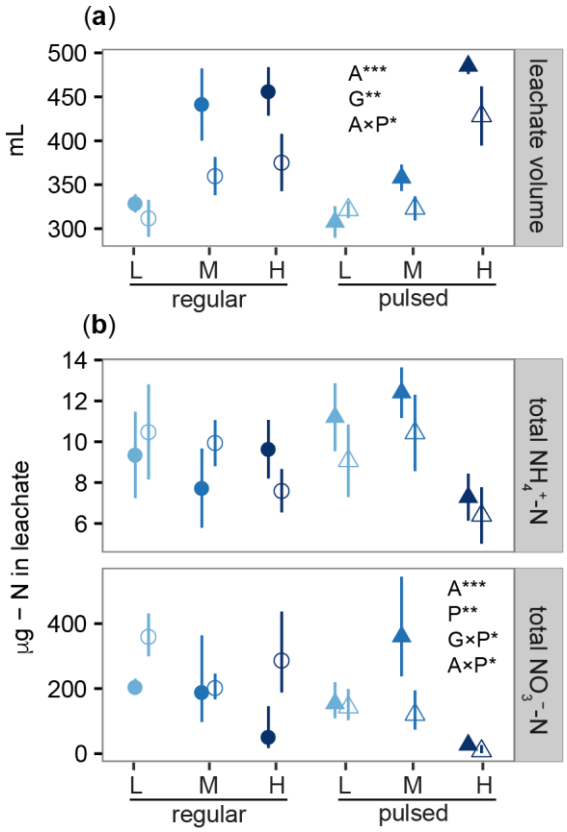

Fig. 5: Leachate volume and total amount of NH_4^+-N and NO_3^--N in leachate following simulated leaching event after growth of AM (MYC+) and non-AM (*rmc*) tomato genotypes grown under varying amounts (low, medium and high) and patterns (regular and pulsed) of water supply. For legend showing water treatments, see Fig. 1. Closed symbols are MYC+, and open symbols are *rmc* plants. On x-axis, L=low, M=medium, and H=high water amounts. Inset in each panel are results of three-way ANOVA (A=amount; G=genotype; P=pattern). For ANOVA results, see Table 1. *p < 0.05; **p < 0.01; *** p < 0.001.

Discussion

This study shows that AM fungi modulate how plants respond to differing water regimes, not only differing in water amounts but also in pattern, including lower and more variable water supplies, as might be expected with climate change. Association with AM fungi increased tomato P uptake with a pulsed water supply, and also increased shoot N concentration with lower water amounts, possibly due to an increased reliance on the AM pathway for nutrient uptake when soil is dry and/or following bursts of nutrient availability during wet/dry cycles.

Furthermore, this study adds to the growing evidence that AM fungi can reduce nutrient losses by showing that antecedent water conditions affect the extent to which reductions in NO_3^- leaching occur. Although simulated leaching losses were low across all treatments (<1 kg N ha^{-1}), AM fungi still reduced NO_3^- leaching by 54% in the regular watering regime. With fewer but more intense rain events expected in many regions as climate change progresses, and since most plants form AM, these findings suggest that AM fungi will be important moderators of plant and ecosystem responses to more variable precipitation.

AM formation and effects on plant biomass and nutrient uptake under contrasting water regimes

Comparing the mycorrhiza defective tomato genotype *rmc* with its well-colonized wildtype progenitor MYC+ provided a means of isolating the effects of AM fungi on plant and ecosystem functioning without directly impacting other soil microbes responsible for nutrient cycling (Cavagnaro et al., 2007). Lower root colonization by AM fungi with lower levels of water supply (a ~30% reduction from high to low water) has been shown in some cases, although increases in colonization rates are apparently more typical (see studies reviewed in Augé, 2001). Since AM fungal colonization rates of roots is not necessarily indicative of AM functionality (Hart & Reader, 2002), it is unclear whether the reduction in root colonization observed here would limit AM effects on plant and ecosystem functions.

The uniformity of the growth depression in AM plants across the water treatments indicates that under these experimental conditions, forming AM associations was a short-term cost. The exception was roots in the low/pulsed water regime. Since AM hyphae are often thought to substitute for root functions, it appears counter-intuitive that AM plants had greater root biomass in this treatment. One possible explanation is that AM hyphae mainly substitute for direct root nutrient uptake, not water uptake (Smith & Read, 2008), and so even slightly higher nutrition in

MYC+ plants may have allowed for more root growth to facilitate water uptake in what was likely the most stressful treatment. Growth depressions in other treatments were likely due to adequate soil available soil P (~34 µg P g^{-1} soil) but low soil N availability (as also shown by low shoot N concentrations of <2% and low plant N:P ratios of 3.9–5.5 across all treatments), since AM net benefits are considered maximal with the opposite ratio of nutrients, low P but high N (Johnson, 2010; Johnson et al., 2015). This possibility is further supported by a meta-analysis of experiments with these genotypes which shows that MYC+ typically has slightly greater biomass than *rmc*, based on experiments that were conducted mainly with lower P and higher N availability than this study (Watts-Williams & Cavagnaro, 2014). However, growth depressions observed during early vegetative growth due to AM formation do not necessarily equate to lower biomass or productivity compared to non-AM plants at later growth stages, especially if nutrient uptake is higher during early growth (Li *et al.*, 2005). Further, a prior field study with these same genotypes under 50% deficit irrigation showed similar vegetative biomass throughout the entire growing season but 25% greater fruit yield at maturity driven by greater nutrient uptake and altered physiological processes that limited stress (Bowles *et al.*, 2016a), showing that vegetative biomass may not be a good indicator of fruit production in these genotypes.

The substantially higher P concentration and P content in MYC+ shoots and roots across all water regimes shows that AM fungi are effective at increasing plant P interception at a wide range of soil moisture conditions (Neumann & George, 2004). The effect of AM fungi on root P concentration was especially pronounced in the pulsed water regime, in which soil moisture was more variable and had greater extremes of wet and dry, than the regular water regime, but with similar mean soil moisture. It is possible that in these conditions, the smaller diameter and

417 greater specific length of AM hyphae compared to roots were important to access increasingly
418 small and disconnected water-filled pore spaces to acquire P as soil moisture declined.
419 Moreover, the greater magnitude of wet/dry cycles occurring in the pulsed water regime may
420 have caused bursts of P availability (Bünemann et al., 2013) that could be better exploited by
421 AM hyphae than roots alone, especially if heterotrophic microbial P immobilization were rapid
422 following rewetting (Yevdokimov et al., 2016).

423 The greater concentration of N in MYC+ shoots and roots may either be a result of AM-mediated
424 plant N acquisition, or the result of reduced biomass in MYC+ plants. Previous work with these
425 same tomato genotypes have shown higher N uptake capacity in MYC+ (Cavagnaro et al., 2006,
426 2012), suggesting the former is a possibility here. Previous work also identified AM-specific
427 NH_4^+ transporters in MYC+ roots that were expressed mainly under low N conditions (Ruzicka
428 et al., 2011), suggesting an increasing reliance on the AM-pathway for N uptake when soil N
429 availability is low. Furthermore, the amount of water supplied affected the mycorrhizal response
430 for shoot and root N concentration, i.e. the difference between AM and non-AM genotypes, more
431 strongly than the mycorrhizal response for shoot and root biomass, suggesting that the AM
432 pathway for plant N uptake is affected by soil moisture. Since AM increased shoot N
433 concentration more at lower levels of water, AM may improve plant N uptake when N is less
434 mobile in soil, as has been shown by others (Tobar & Barea, 1994; Subramanian & Charest,
435 1999) and suggested as a plausible context when AM could improve plant N acquisition (Smith
436 et al., 2009). Yet in roots, the effect of AM on N concentration was least at the lowest water
437 level, suggesting that AM may also be affecting the partitioning of N into above- vs. below-
438 ground biomass, perhaps to maximize C gain in leaves.

Lower biomass and, we assume, lower evapotranspiration in MYC+ plants likely lead to the slightly higher soil moisture observed in MYC+ microcosms under the regular water regime (Fig. 1). Lower soil moisture, rather than a direct influence of AM on soil and plant processes, could be responsible for the some of the plant nutrient responses attributed to AM. We evaluated this possibility by plotting shoot and root N and P concentrations at final harvest as a function of mean soil moisture during the treatment period for MYC+ and *rmc* plants separately (Fig. S1). If soil moisture were the main driver of plant nutrient responses, then we would expect to see similar slopes and/or intercepts for both genotypes, but this is not the case for any response variable. As an example, the intercept for shoot N concentration of MYC+ plants is 1.70 vs 1.49 for *rmc*, while the slope is lower (0.44 vs. 0.92, respectively), which reflects higher N concentration in MYC+ plants mainly at lower soil moisture. While small differences in soil moisture do exist between the genotypes, it appears that a direct effect of AM on plant and soil processes rather than an indirect effect of growth depressions on soil moisture are primarily driving the plant nutrient responses.

AM effects on N uptake and N leaching following contrasting water regimes

Although AM plants typically had higher N concentrations and similar N content, we did not observe higher recovery of the $^{15}NH_4^+$ pulse in AM plants during the four-day period at the end of the experiment as we had expected. The slightly higher ^{15}N recovery in *rmc* shoot and roots may be because MYC+ had higher N concentration prior to the N pulse, resulting in lower N demand over the short-term period. It is also possible that AM plants were relying more on the AM pathway for uptake of existing soil N, and the newly added N was not a part of this source. Prior research with these genotypes also showed a trend toward higher ^{15}N recovery in *rmc* shoots over 24 hours following a pulse of $^{15}NH_4^+$ under well-watered conditioned in the field

462 (Ruzicka et al., 2011), with a similarly low recovery of the N pulse in shoots and roots (7% of
463 applied ^{15}N vs. 4% in this study). Recovery was low likely because the pulse of ^{15}N was large
464 (equivalent to 100 kg N ha^{-1}) and the time period of recovery was relatively short (4 days). It is
465 also possible that some of the ^{15}N was lost via denitrification, especially in the high water
466 amount treatment when water filled pore space reached ~65% (Linn & Doran, 1984). In this
467 study, higher ^{15}N recovery at higher amounts of water was likely because plants were larger and
468 had greater N demand due to reduced water stress, and because greater amounts of water allowed
469 for more movement of N down through the microcosm and thus across more roots. Indeed we
470 observed much less residual soil NH_4^+ in surface soil (0–9 cm depth) at antecedent high levels of
471 water compared to lower levels (9.1 vs. 24.5 µg NH_4^+-N g^{-1} soil in low vs. high water treatments,
472 respectively).

473 The role of AM fungi for increasing N retention has received increasing attention (van der
474 Heijden, 2010; Asghari & Cavagnaro, 2012; Bender et al., 2015; Cavagnaro et al., 2015; Köhl &
475 van der Heijden, 2016), with some studies showing large effects of AM fungi on reducing
476 simulated NO_3^- leaching that could not be attributed to size asymmetry between AM and non-
477 AM plants (Asghari & Cavagnaro, 2012), but others showing no effect (Bender et al., 2015).
478 Reductions in NO_3^- leaching with AM present have been attributed to enhanced plant N
479 interception, N immobilization in AM fungal biomass, improvements in soil structure and
480 subsequent reductions in leachate volume, and as yet unknown effects on other soil microbes
481 responsible for soil N transformations (Bender et al., 2015; Cavagnaro et al., 2015). In this study,
482 the 54% reduction in NO_3^- leaching in AM microcosms under the regular water regime cannot be
483 attributed to higher N uptake in MYC+ plants (similar total N content), or reductions in leachate
484 volume, which were actually higher in AM microcosms due to higher antecedent soil moisture.

485 This points to a more subtle effect of AM fungi on NO_3^- leaching, possibly by increasing plant
486 uptake of NH_4^+ vs. NO_3^- and thus leaving less NH_4^+ available for nitrification, rather than a
487 direct effect on ammonia oxidizers (Cavagnaro et al., 2007). Since NH_4^+ is less mobile than NO_3^-
488 and NH_4^+ is the form of N transferred to plant roots (Govindarajulu et al., 2005), it is thought
489 that AM fungi may increase plant uptake of NH_4+ more than NO_3^-. The influence of genotype on
490 surface soil NO_3^- concentrations prior to leaching, in concert with both the amount and pattern of
491 water, does suggest complex and interacting effects of AM fungi and water supply on soil N
492 forms, with possible downstream effects on ecosystem processes like NO_3^- leaching. Since NO_3^-
493 leaching was only lower in MYC+ plants at low and high amounts of water in the regular water
494 regime, the underlying mechanism may also vary depending on soil moisture, given its strong
495 influence on all soil and microbial N processes affecting nitrate pools (Porporato *et al.*, 2003). If
496 soil wet/dry cycles caused higher cumulative gaseous N losses in the pulsed water treatment
497 compared to more consistent soil moisture conditions in the regular treatment (Borken &
498 Matzner, 2009), then it may explain why NO_3^- leaching losses were generally lower in the pulsed
499 treatments. The small absolute difference in NO_3^- leaching between MYC+ and *rmc* genotypes in
500 the regular water treatment (only 0.25 kg N ha^{-1}), suggests that the majority of the 100 kg N ha^{-1}
501 $^{15}NH_4^+$-N pulse added 4 days prior likely remained in soil, possibly immobilized by N-limited
502 microbes or remaining as NH_4^+ and held by negatively-charged sites in soil.

503 Changing precipitation patterns, especially fewer but more intense rainfall events and summer
504 droughts, could increase N losses in agricultural landscapes where tomato and other N-intensive
505 crops are grown (Robertson et al., 2013; Gelfand et al., 2015; Loecke et al., 2017). Higher N
506 losses result in part from increased crop stress when water is limiting, which reduces N uptake
507 and leaves large amounts of residual N in soil (Gentry et al., 1998) that can be lost via leaching

or denitrification. This study, in concert with other recent work (Lazcano et al., 2014; Bowles et al., 2016a), reinforces the potential importance of AM fungi for reducing the impacts of low and more variable water availability on plant performance, nutrient uptake, and N losses. This points to the need for more targeted research to unravel when and how managing AM and other plant-microbe interactions will be most effective to boost ecosystem services like nutrient retention (Bender et al., 2016).

In summary, AM fungi affected plant growth and nutrient acquisition not only under low water, but also when water supply is more variable. We also show that AM fungi modulate the extent to which antecedent soil moisture patterns affect other ecosystem services like nutrient retention, building on recent work on this under-recognized role for AM fungi (Cavagnaro et al., 2015). Though changes in precipitation patterns strongly affect plant growth and ecosystem processes, the influence of AM fungi has not previously been studied. This study provides a first step in understanding a potentially important role for AM fungi in modulating these responses, and underscores the need for more studies to elucidate the mechanisms involved, especially in field conditions. In managed ecosystems, this also presents an opportunity to use management strategies that bolster AM communities (Lekberg & Koide, 2005; Lehman et al., 2012; Bowles et al., 2016b) and possibly create systems that are more resilient to a more variable climate expected in many regions of the world.

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