Title

Soil moisture legacy effects: impacts on soil nutrients, plants and mycorrhizal responsiveness.

Author
Timothy R Cavagnaro

Affiliation

[1]School of Agriculture, Food and Wine, University of Adelaide, Waite Campus, PMB1

Glen Osmond, SA, 5064, Australia.

Email: Timothy.cavagnaro@adelaide.edu.au

Phone: +61 8 8313 2770

Keywords

Climate Change; Drought; Field Capacity; Soil Ecology, Legacy Effect

Abstract

Although most land-plants form associations with arbuscular mycorrhizal fungi (AMF) as a means of optimising nutrient capture, legacy effects of altered soil moisture regimes on plant responses to arbuscular mycorrhizas (AM) have not been studied. As rainfall patters change with climate changes, soil moisture legacy effects, and their impact on plants, soil and microbes may become increasingly important. Results of an experiment are presented in which soil was subjected to a range of different soil moisture regimes prior to planting a mycorrhiza-defective tomato mutant and its mycorrhizal wild-type progenitor. There were clear legacy effects of the soil moisture regime prior to planting on soil physicochemical properties, plant growth and nutrition, the formation of AM and mycorrhizal responsiveness. For example, in the Dry treatment the plants were well colonized by AM, there was a clear benefit to the plants in terms of mycorrhizal growth responses and mycorrhizal P responses. In contrast, in the Intermediate treatment AM colonisation was lower, there was little benefit in terms of mycorrhizal responses. Finally, in the Wet and Wet/Dry treatments AM colonization levels were similar to those in the Dry treatment, but mycorrhizal growth responses were lower and more variable. Together, these results clearly indicate that soil nutrients, plant growth and nutrition and mycorrhizal responsiveness are affected by soil moisture legacy effect. Consequently, as we move into a period where more variable and intense rainfall amounts and patterns have been projected, we need to consider soil moisture legacy effects.

Introduction

Climate models are projecting a drier and/or a more variable (in terms of rainfall) climate for many regions of the world (IPCC, 2013; Jentsch et al., 2007). More frequent extreme weather events associated with climate change (IPCC, 2013; Jentsch et al., 2007) are expected to increase abiotic and biotic stress on plants. In addition to the direct impacts of changes in the amount, timing and intensity of rainfall events on plants, indirect impacts can also occur (Knapp and Smith, 2001). For example, nutrient availability and soil microbial community composition, both of which affect plant growth (Bardgett and Wardle, 2010; van der Heijden et al., 1998), can change in response to soil moisture (Franzluebbers et al., 1994; Meisnera et al., 2013) (Brockett et al., 2012; Drenovsky et al., 2010). These indirect effects can result in the establishment of "soil moisture legacy effects" where plants are impacted by conditions prior to plant establishment (Meisnera et al., 2013).

Plants have evolved many strategies and traits for optimising nutrient acquisition (Lynch, 2007), including the formation of arbuscular mycorrhizas (AM) (Lambers et al., 2008; Smith and Read, 2008). Under nutrient limiting conditions, the formation of AM can increase plant fitness and competitiveness, which has important consequences for ecosystem productivity and biodiversity (Cavagnaro et al., 2004; Facelli et al., 1999; van der Heijden et al., 1998). Although most land-plants form AM, soil moisture legacy effects (Meisnera et al., 2013) on the formation of AM and plant responses to arbuscular mycorrhizal fungi (AMF) have not been studied.

Although the impact of soil moisture legacy effects on AM formation and functioning remain unknown, some predictions can be made. For example, the wetting of soils in the absence of plants may trigger germination of spores of AMF,

65 but in the absence of a suitable host plant, this may see a reduction in the inoculum
66 potential of the soil (Giovannetti et al., 2002). Thus, a consequence of soil moisture
67 legacy effects may be a reduction in the colonisation of roots by AMF. Additionally, if
68 soil moisture legacy effects result in a reduction in soil nutrient availability (e.g. via
69 stimulation of denitrification under wet conditions leading to gaseous soil N loss),
70 the relative benefit of forming AM may be higher. Conversely, if soil moisture legacy
71 effects result in an increase in plant-available nutrients (e.g. via stimulation of
72 mineralization N and P), the role of AM may be diminished. Taken together, a
73 consequence of soil moisture legacy effects on AM may be a change in the balance
74 between the costs and benefits of forming AM, with shift from negative, neutral or
75 positive mycorrhizal responses resulting (Johnson et al., 1997).
76 Since most plants form AM, and these associations can have a major impact
77 on plant growth and nutrient acquisition, the impact of soil moisture legacy effects
78 on the formation and functioning of AM could potentially be very significant. Here,
79 are presented results of a study testing the hypothesis that a history of dry, wet,
80 intermediate or variable (wet/dry cycles) soil moisture conditions prior to planting
81 will affect the formation and functioning (in terms of impacts on plant nutrition and
82 growth) of AM, due to changes in soil nutrient availability. The experiment involved
83 growing a mycorrhiza defective tomato mutant, and its mycorrhizal wildtype
84 progenitor (Barker et al., 1998) in soils with (experimentally established) different
85 soil moisture legacies. This genotypic approach for controlling the formation of AM
86 was selected as it allows for the comparison of mycorrhizal and non-mycorrhizal
87 plants with the wider soil biota intact (Rillig, 2004; Watts-Williams and Cavagnaro,

88 2015), and because the two genotypes exhibit very similar growth patterns when

89 grown in the absence of AMF (Watts-Williams and Cavagnaro, 2014).

Materials and methods

The soil used in this experiment was an Urrbrae red-brown earth (Alfisol), collected from the 0-10 cm soil layer of The University of Adelaide's Waite Campus Arboretum, South Australia, in April 2014 (Austral Autumn). This soil was selected as it has previously been shown to have high levels of AM inoculum potential and provides a good growth medium for our model plant, tomato (see below). The soil was air-dried and sieved to <2 mm prior to use to homogenise the soil and remove rocks and coarse woody debris. The soil has a pH (1:5 soil:water extract) of 6.3 ± 0.01 and a total C concentration of 4.7 ± 0.3%. The NH_4^+-N concentration of the soil, which was measured colorimetrically on 2M KCl extracts (Forster, 1995), was 7.3 ± 0.2 (µg/g dry soil), and the NO_3^--N concentration, also measured colorimetrically on 2M KCl extracts (Miranda et al., 2001), was 3.1 ± 0.1 (µg/g dry soil). The plant-available (Colwell) P concentration of the soil was 3.0 ± 0.04 (µg/g dry soil). The field capacity of the soil was determined using a sintered glass funnel connected to a 100 cm water column (Ψ_m = −10 kPa). Soil was packed in the glass funnel to the same bulk density as the field site from which it was collected (1.36 g/cm^3), saturated with water and allowed to drain for 48 hrs and weighed. The soil was then dried at 105 °C for 48 hr and gravimetric moisture content calculated. The gravimetric moisture content at field capacity was 0.35 g water/g dry soil.

To each of 40 plastic bags was added 884 g of dry soil. Reverse Osmosis (RO) water was then added to the bags in varying amounts to establish the following four soil moisture treatments (i.e. 10 bags per treatment). Dry treatment: water added to 25% of water holding capacity (gravimetric moisture content of 0.9 g water/g dry soil). Intermediate treatment: water added to 50% of water holding capacity

114 (gravimetric moisture content of 0.18 g water/g dry soil). Wet treatment: water
115 added to 75% of water holding capacity (gravimetric moisture content of 0.27 g
116 water/g dry soil). Wet/Dry treatment: water added to 75% of water holding capacity
117 (gravimetric moisture content of 0.27 g water/g dry soil). These moisture contents
118 were selected as 75% of water holding capacity provides optimal conditions for plant
119 growth in the soil, and 25% of water holding capacity can be achieved when the soil
120 is left to dry under typical glasshouse conditions in a reasonable amount of time
121 (preliminary data not shown, but see Figure 1). N.B. the Wet/Dry treatment was
122 subjected to drying and re-wetting later in the experiment, as outlined below.
123 Immediately following the addition of water to the soil in the bags the soil was mixed
124 thoroughly to ensure an even distribution.

125 One day after water was added to the soil in the plastic bags, the soil was
126 transferred to plastic, non-draining pots. These pots were then moved to a
127 glasshouse facility on the Waite campus where they remained for the remainder of
128 the experiment. Conditions in the glasshouse were set to 22-26^{o}C and daytime light
129 levels, with supplemental lighting were 950 µmol m^{-2} s^{-1} with a 16/8 day/night
130 photoperiod. The pots in the Dry, Intermediate and Wet treatments were weighed
131 thrice weekly and water added to the pots to maintain them at their target moisture
132 content for a period of 93 days (Figure 1). Pots in the Wet/Dry treatment were also
133 weighed thrice weekly and water loss (by mass) recorded; however, in this
134 treatment, they were maintained at 75% of water holding capacity (by adding water)
135 for 14 days, at which time watering was ceased until the soil reached 25% of water
136 holding capacity (35 d). From day 35-45 the pots were maintained at 25% of water
137 holding capacity by adding water as required. On day 45 the pots were then re-

138 watered up to 75% of water holding capacity and maintained at this moisture
139 content until day 49. On day 49 watering was again ceased until the soil reached 25%
140 of water holding capacity (73 d). From day 73-82 the pots were maintained at 25% of
141 water holding capacity. On day 82 the pots were then re-watered up to 75% of water
142 holding capacity and maintained at this moisture content until day 94 (see Figure 1).
143 On day 94, all pots in all treatments were watered up to 75% of water
144 holding capacity, and seedlings planted into the pots on the same day, as follows. In
145 the middle of each pot a small soil core was taken (approx. 10 g) using a 10 mm
146 diameter stainless steel cork borer. The soil from the core was analysed for
147 concentrations of NH_4^+-N, NO_3^--N and plant available (Colwell) P, as described above.
148 Into each hole one three week old tomato seedling (or either of two different
149 genotypes, as follows) was planted. The tomato genotypes were a reduced
150 mycorrhiza colonisation tomato (*Solanum lycopersicum* L.) mutant genotype (*rmc*,
151 hereafter), and its AM mycorrhizal progenitor (76R, hereafter) (Barker et al., 1998).
152 The seedlings were raised by surface-sterilising the seeds, pre-germinating them on
153 moist filter paper for 5 days (following Cavagnaro et al., 2010), and sowing the seeds
154 into individual seed raising containers, each containing approx. 50 g of sterile sand.
155 The seedlings were transplanted by gently washing them from the sand in which
156 they were sown and then placing them in the hole created in the centre of each pot.
157 The small void surrounding the roots of the seedlings was then gently backfilled
158 using sterile sand. Immediately after planting, all pots were watered to 75% of water
159 holding capacity, at which moisture content they were maintained for the remainder
160 of the experiment.

Thirty-seven days after the seedlings were transplanted into the pots, all plants were destructively harvested; this time was selected as plants have had sufficient time for roots to be colonised by AMF and have not begun to senesce. The plants were carefully washed from the soil with RO water. All the shoots and a sub-sample of the roots were oven-dried (50 ^{o}C) until a constant mass was achieved, and dry weights determined. The dried plant material was then ground to a fine powder and P concentrations determined by radial view inductively coupled plasma-optical emission spectroscopy (Waite Analytical Services, , Urrbrae, South Australia) and N concentrations by catalytic combustion and thermal conductivity (vario MICRO cube, Elementar Analysensysteme GmbH, Hanau, Germany). The second root sample was used for assessment of mycorrhizal colonisation using the gridline intersect method (Giovannetti and Mosse, 1980), after roots were cleared with KOH (10% W/V) (Phillips and Hayman, 1970) and stained with ink and vinegar (Vierheilig et al., 1998).

Data calculations and analysis

Mycorrhizal growth responses (MGR) were calculated following Eqn 1.

$$\%MGR = \frac{dry\ weight\ (76R) - mean\ dry\ weight\ (\mathrm{rmc})}{mean\ dry\ weight\ (\mathrm{rmc})} \times 100 \qquad \text{Eqn 1}$$

To further explore patterns of P and N uptake by mycorrhizal and non-mycorrhizal plants on a tissue content basis (i.e. not per g of tissue), whole plant tissue contents were used to determine the Mycorrhizal P uptake Response (MPR) and Mycorrhizal N uptake Response (MNR) (following Cavagnaro et al., 2003). The Mycorrhizal P

185 Response was calculated using the individual P content of 76R plants and mean P
186 content of *rmc* plants: Eqn 2.
187
188 $$\%MPR = \frac{P\ content\ (76R) - mean\ P\ content\ (\mathrm{rmc})}{mean\ P\ content\ (\mathrm{rmc})} \times 100 \qquad \text{Eqn 2}$$
189
190 The Mycorrhizal N Response was calculated in the same manner as the MPR (see
191 Eqn. 2).
192
193 Over the course of the experiment, two 76R plants in the intermediate
194 watering treatment, and one *rmc* plant in the Wet/Dry treatment, died after
195 transplanting. These plants showed signs on a foliar pathogen; these plants were
196 separated from all other plants and no other plants developed these symptoms.
197 These replicates were omitted from all data analysis; therefore, *N*=8-10 for soil
198 analyses and *N*=3-5 for plant analyses, as indicated in the figure captions.
199 Data on soil properties at the time of planting between watering treatments
200 were compared by one-way GLM. Where the analysis revealed a significant pairwise
201 comparisons were made between treatments using Tukeys HSD test. Data on plant
202 dry weights and nutrient contents at the time of planting were compared between
203 watering treatments and genotypes by two-way GLM. The factors in the analysis
204 were *Genotype* and *Watering Treatment*. Where the analysis revealed a significant
205 difference (*P<0.05*) pairwise comparisons were made between treatments using
206 Tukey's HSD test. For data on mycorrhizal colonisation of roots (%), only data for the
207 76R genotype were analysed (by one-way GLM, with pairwise comparisons by
208 Tukey's HSD) as levels of colonisation in the *rmc* genotype were <1.5% and were

209 therefore omitted from this analysis. All data were analysed using JMP statistical

210 software (version 11.0.0).

211

Results

Soil properties

Altering the supply of water to the soil during the 95 days prior to planting of the seedlings significantly altered soil physicochemical properties. The initial concentrations of NH_4^+-N, NO_3^--N and plant available (Colwell) P in the soil when collected from the field were lower, higher and lower, respectively than at the time of planting. At the time of planting, although the soils were generally low in NH_4^+-N, concentrations were significantly higher in the Wet/Dry treatment compared to all other treatments (Figure 2a). The concentration of NO_3^--N in the soils was very high, but especially so in the Wet and Wet/Dry treatments compared to the Intermediate and Dry treatments (Figure 2b). Total mineral N (NH_4^+-N plus NO_3^--N) followed the same pattern as NO_3^--N (data not shown, but compare Figures 2a and b). Although plant available P (Figure 2c) was low in the soil, there was a small difference between treatments, with P increasing between the watering treatments in the order Dry<Wet<Wet/Dry<Intermediate.

Mycorrhizal colonisation, plant growth and nutrients

The formation of AM was significantly affected by the supply of water to the soils prior to planting. Specifically, the per cent of root length colonized was significantly lower in the Intermediate watering treatment compared to the Dry and Wet treatments (Figure 3a). Levels of colonisation in the Wet/Dry treatment were intermediate to those in the Wet and Intermediate watering treatments and lower than in the Dry treatment. Root length did not differ significantly among any of the

235 treatments (data not shown), and so mycorrhizal root length followed a similar
236 pattern as per cent root length colonised (data not shown).
237 The growth of plants was significantly affected by the watering regime prior
238 to planting. For shoot dry weight (SDW) (Figure 3b) this response was modulated by
239 the formation of AM, as indicated by a significant two-way interaction between
240 watering treatment and genotype in the analysis. Specifically, the SDW of plants in
241 the intermediate watering treatment were highest, and did not differ between the
242 genotypes. Further, whereas the growth of 76R plants (i.e. formed AM) in the Dry
243 treatment was similar to that of those in the Intermediate treatment, but those of
244 the *rmc* genotype (i.e. did not form AM) in the Dry treatment were significantly
245 smaller. Plants in the Wet treatment followed a similar trend, but the difference in
246 SDW between 76R and *rmc* plants was not statistically significant. For the Wet/Dry
247 treatment, there was no difference in the SDW of the mycorrhizal and non-
248 mycorrhiza plants, and those plants were generally smaller than in the other
249 treatments. Plant growth below ground (root dry weight (RDW), Figure 3c) was
250 generally unaffected by the formation of AM. However, when each of the
251 treatments were considered separately (targeted t-tests), the 76R plants in the Dry
252 treatment were found to have a significantly a higher RDW compared to their *rmc*
253 counterparts (see Figure 3c). In contrast, prior soil moisture regime had a significant
254 impact on RDW (irrespective of genotype), with the RDW of plants increasing
255 between treatments in the order Dry<Wet/Dry<Wet<Intermediate. When
256 mycorrhizal growth responses were calculated on a whole plant basis (i.e. SDW plus
257 RDW), there was a positive MGR in the Dry treatment (26 ± 2%), a small (albeit
258 variable) positive MGR in the Wet (14 ± 9) and Wet/Dry (14 ± 9%) treatments, and

no MGR in the Intermediate treatment (-2 ± 1%); Although there was no significant difference in MGRs due to the high level of variation in two of the treatments, it is clear that in the Dry and Intermediate treatments there were positive and neutral MGRs; the difference between these two treatments when compared in a separate analysis was significant (*P<0.0001*).

Although there were only small differences in soil P concentrations among the watering treatments, there was a clear soil moisture legacy effect on plant P nutrition. The concentration of P in the shoots of 76R plants (Figure 4a) was significantly higher than their *rmc* counterparts, with the exception of the Intermediate watering treatment there was no difference between the genotypes. A similar pattern was seen in root P concentrations (Figure 4b), with the exception of the Wet/Dry treatment, where the concentrations were not significantly different between the genotypes. When MPRs were calculated on a whole plant (i.e. root plus shoot) P content (mg/plant) basis, there was a strong positive MPR in all treatments, with the exception of the Intermediate watering treatment (MPR (%): Dry = 85 ± 4^{c}; Intermediate = 10 ± 1^{a}; Wet 57 ± 6^{b}; Wet/Dry = 42 ± 8^{b}. N.B. Treatment P<0.0001, means ± S.E followed by the same superscript letter are not significantly different at the *P<0.05* level).

The concentration of N in the shoots (Figure 4d) of plants was marginally higher in the Wet and the Wet/Dry treatments than in the other treatments. The only significant difference in shoot N concentrations between the genotypes was in the Dry treatment where the *rmc* plants had a higher concentration than the 76R plants. There were no differences in root N concentrations (Figure 4e). However, when MNRs were calculated there were clear positive MNRs in the Wet and

283 Wet/Dry treatments (MNR(%): Dry = -2.2 ± 1.4^{b}; Intermediate = 1.5 ± 4^{b}; Wet 29.7 ±

284 5.9^{a}; Wet/Dry = 10.6 ± 2.9^{b}. N.B. Treatment P<0.001, means ± S.E followed by the

285 same superscript letter are not significantly different at the *P<0.05* level).

286

Discussion

There were clear legacy effects of the soil moisture regime prior to planting on soil physicochemical properties, plant growth and nutrition, the formation of AM and mycorrhizal responsiveness. The reasons underlying these changes and their potential implications in the context of a drying climate, are discussed.

Soil moisture legacy effect: soil properties

Impacts on soil nutrients are an important component of soil moisture legacy effects. Changes in soil moisture had a significant impact on soil mineral N availability as expected (Burger et al., 2005; Franzluebbers et al., 1994; Yu and Ehrenfeld, 2009). Although starting levels of NH_4^+-N in the soil were low (7.3 μg/g), they were even lower at the time of planting (<0.1 - 4 μg/g). This decrease in NH_4^+-N suggests that rates of nitrification and/or microbial N immobilization were greater than rates of ammonification (Cavagnaro et al., 2008). The decrease in NH_4^+-N at the end of the first phase of the experiment was smallest in the wet-dry treatment, and may reflect lower levels of nitrification and/or microbial immobilization, and/or higher levels of ammonification in this treatment. In support of this, pervious work has shown that nitrification can be lower with wet/dry cycles compared to continuous moisture (Xiang et al., 2008). In direct contrast to NH_4^+-N, soil NO_3^--N concentrations were much higher than at the time of planting (approx. 10-100 fold) in all, and especially the Wet and Wet/Dry, treatments. The large build up of NO_3^--N was likely due to high rates of N mineralization releasing large amounts of NH_4^+-N, which was then nitrified (to NO_3^--N), but not subsequently denitrified (to N_2O or N_2) as all soils were aerobic (Burger et al., 2005; Xiang et al., 2008). Thus, soil moisture

311 legacy effects on mineral N pools may vary on the extent to which soil aerobicity and
312 anaerobicity are impacted.
313 The soil moisture legacy effect also extended to impacts on plant available P
314 in the soil, with available P concentrations higher at the time of planting than in the
315 starting soil, consistent with earlier work (Austin et al., 2004). At the time of
316 planting, available P concentrations were lowest in soil from the Dry treatment and
317 highest in the Intermediate treatment (although differences were small), where
318 mycorrhizal colonisation and responsiveness were greatest and lowest, respectively
319 (see below). This is in contrast to an earlier study in which there was no P release,
320 nor change in AM colonisation, in soils subjected to different watering regimes,
321 albeit over a much shorter time frame (Cui and Caldwell, 1997).
322 Although not the focus of this experiment, soil moisture legacy effects will
323 almost certainly extend to impacts on the biomass, activity and potentially the
324 diversity, of soil microbial communities (beyond AMF), as in earlier work on soil
325 moisture legacy effects (Meisnera et al., 2013). It is likely that such changes in
326 microbial communities will include microbes involved in soil nutrient cycling such as
327 ammonia oxidisers, denitrifiers, P-solubilizers and others (Li et al., 2008; Xiang et al.,
328 2008). Given the important role of soil microbes in the release of nutrients from soil
329 organic matter, it will be important to investigate the link between soil moisture
330 legacy effects and microbial communities, beyond AMF alone.
331
332 *Soil moisture legacy effect: mycorrhizal formation and responsiveness*
333 The soil moisture legacy effect included impacts on the formation and functioning of
334 AM. As noted above, AM colonisation was highest in plants in the Dry treatment

where soil P was lowest, and lowest in the Intermediate treatment were soil P was highest. Levels of colonization in the Wet and Wet/Dry treatments were intermediate. Although it is well established that increasing soil P availability can reduce the colonisation of roots by AMF (Baon et al., 1992; Bolan et al., 1984), the differences in soil P here were very small and unlikely to have caused a more than halving of AM colonisation. There was no clear impact of increasing N availability on AM colonisation, in contrast to results of a meta-analysis showing general increase in AM colonisation with N fertilization (Treseder, 2004). Thus, the variation n AM colonization may be due to other factors.

Wetting of dry soil in the absence of a plant can reduce the inoculum potential of the soil, due to spores of AMF germinating in the absence of a suitable root system to colonize (Giovannetti et al., 2002). However, this appears not to have been the case here as levels of AM colonisation were in the typical range for this tomato genotype (Asghari and Cavagnaro, 2012; Cavagnaro et al., 2012; Watts-Williams and Cavagnaro, 2014). Further, if the legacy effect was due to a reduction in soil inoculum potential associated with spores of AMF germinating but not finding a suitable host after a soil wetting event, we would have predicted the lowest level of colonisation in Wet/Dry treatment which included two complete wet/dry cycles in the absence of a plant; this however, was not the case. Furthermore, the complexity of soil moisture legacy effects is highlighted by the fact that levels of AM colonization were equally high in the wet and dry treatments. While the reason for this is unknown, it suggests that any impact of soil moisture legacy effects on the inoculum potential of the soil is not driven by a simple linear gradient in soil moisture.

359 Mycorrhizal responsiveness - the biomass of mycorrhizal plants relative to
360 that of their non-mycorrhizal counterparts - was strongly influenced by the soil
361 moisture legacy effect. For example, a positive mycorrhizal growth response was
362 observed in the Dry treatment where soil P was marginally lower and mycorrhizal
363 colonisation was greatest, and no mycorrhizal response was observed in the
364 intermediate treatment where in soil P was highest and mycorrhizal colonisation
365 lowest. Furthermore, the biomass of the mycorrhizal plants in the Dry treatment was
366 equivalent to that of the mycorrhizal and non-mycorrhizal plants in the intermediate
367 treatment. Mycorrhizal growth responses and MPRs were also positive in the Wet
368 and Wet/Dry treatments, where again soil P was slightly (but significantly) lower
369 than in the intermediate treatment. Thus, it appears that the formation of AM
370 following dry, and to a lesser extent wet and wet/dry, conditions can help to
371 overcome (albeit small) soil moisture legacy effects on soil available P. This may have
372 important implications in the context of current projections of a drying climate in
373 many regions of the world (IPCC, 2013; Jentsch et al., 2007) and increasing scarcity of
374 readily mined P fertilizers (Cordell et al., 2009). That AM were of greater benefit
375 following the extremes of soil moisture tested in this experiment (i.e.Dry and Wet
376 treatments) in the pre-incubation phase before planting suggests that the
377 importance of AM may be increased as our climate become more extreme and/or
378 variable. While this result is in need for further investigation, it is certainly intriguing.
379 The large changes in mineral N in the soil, especially NO_3^--N, were not
380 associated with clear patterns of change in N in the tissues of the plants. The largest
381 differences in plant N concentrations were seen between the mycorrhizal and non-
382 mycorrhizal plants in the Dry treatment. However, when the relatively larger

biomass of the mycorrhizal plants in this treatment were taken into account (i.e. to calculate MNR on a whole plant N content basis) there was no difference in the capacity of the mycorrhizal and non-mycorrhizal plants to acquire N from the soil. Interestingly, there was a positive MNR in the Wet treatment where soil NO_3^--N was high and there was very little NH_4^+-N, but less so in the Wet/Dry treatment where NO_3^--N was also high but there was more NH_4^+-N. The higher MNR in the Wet/Dry than the Wet treatment may be due to the fact that AMF preferentially take up N as NH_4^+-N over NO_3^--N (Tanaka and Yano, 2005).

Soil moisture legacy effect and AM: conclusions

Irrespective of the underlying mechanisms, soil moisture legacy effects had an important impact on the formation and functioning of AM. While it is very well understood that AM functioning is affected by the plant and fungal genotypes, and environmental factors during the growth and development of the association (Smith and Read, 2008), this study demonstrates that so too are soil moisture legacy effects. For example, in the Dry treatment the plants were well colonized by AMF, there was a clear benefit to the plants in terms of MGR and MPR. In contrast, in the Intermediate treatment AM colonisation was lower, there was little benefit in terms of MGR and MPR. Thus, as we consider the costs and benefits of forming AM, especially as we move into a period of significant climate change, we need to consider legacy effects.

Acknowledgements

The author gratefully acknowledges Dr Vanessa Carne-Cavagnaro and Ms Rebecca Stonor for assistance in conducting this experiment. Thanks also to Prof. Sally Smith and Prof. Andrew Smith for valuable discussions over the years, and to the journal editor and three anonymous reviewers for their constructive and helpful comments. I also gratefully acknowledge the Australian Research Council for supporting my research via the award of a Future Fellowship (FT120100463). Excelsior!

413 **References**
414
415 Asghari, H.R., Cavagnaro, T.R., 2012. Arbuscular mycorrhizas reduce nitrogen loss via
416 leaching. PLoS One 7, e29825.
417 Austin, A.T., Yahdjian, L., Stark, J.M., Belnap, J., Porporato, A., Norton, U., Ravetta,
418 D.A., Schaeffer, S.M., 2004. Water pulses and biogeochemical cycles in arid and
419 semiarid ecosystems. Oecologia 141, 221-235.
420 Baon, J.B., Smith, S.E., Alston, A.M., Wheeler, R.D., 1992. Phosphorus efficiency of
421 three cereals as related to indigenous mycorrhizal infection. Aust. J. Ag. Res. 43, 479-
422 491.
423 Bardgett, R.D., Wardle, D.A., 2010. Aboveground-Belowground Linkages: Biotic
424 Interactions, Ecosystem Processes, and Global Change. Oxford University Press,
425 Oxford.
426 Barker, S.J., Stummer, B., Gao, L., Dispain, I., O'Connor, P.J., Smith, S.E., 1998. A
427 mutant in Lycopersicon esculentum Mill. with highly reduced VA mycorrhizal
428 colonization: isolation and preliminary characterisation. Plant J. 15, 791–797.
429 Bolan, N.S., Robson, A.D., Barrow, N.J., 1984. Increasing phosphorus supply can
430 increase the infection of plant roots by vesicular-arbuscular mycorrhizal fungi. Soil
431 Biol. Biochem. 16, 419-420.
432 Brockett, B.F.T., Prescott, S.J., Grayston, S.J., 2012. Soil moisture is the major factor
433 influencing microbial community structure and enzyme activities across seven
434 biogeoclimatic zones in western Canada. Soil Biol. Biochem. 44, 9-20.
435 Burger, M., Jackson, L.E., Lundquist, E.J., Louie, D.T., Miller, R.L., Rolston, D.R., Scow,
436 K.M., 2005. Microbial responses and nitrous oxide emissions during wetting and
437 drying of organically and conventionally managed soil under tomatoes. Biol. Fert.
438 Soil 42, 109-118.
439 Cavagnaro, T.R., Barrios-Masias, F.H., Jackson, L.E., 2012. Arbuscular mycorrhizas and
440 their role in plant growth, nitrogen interception and soil gas efflux in an organic
441 production system. Plant Soil 353, 181-194.
442 Cavagnaro, T.R., Dickson, S., Smith, F.A., 2010. Arbuscular mycorrhizas modify plant
443 responses to soil zinc addition. Plant Soil 329, 307-313.
444 Cavagnaro, T.R., Jackson, L.E., Hristova, K., Scow, K.M., 2008. Short-term population
445 dynamics of ammonia oxidizing bacteria in an agricultural soil. Appl. Soil Ecol. 40, 13-
446 18.
447 Cavagnaro, T.R., Smith, F.A., Ayling, S.M., Smith, S.E., 2003. Growth and phosphorus
448 nutrition of a *Paris*-type arbuscular mycorrhizal symbiosis. New Phytol. 157, 127-134.
449 Cavagnaro, T.R., Smith, F.A., Hay, G., Carne-Cavagnaro, V.L., Smith, S.E., 2004.
450 Inoculum type does not affect overall resistance of an arbuscular mycorrhiza-
451 defective tomato mutant to colonisation but inoculation does change competitive
452 interactions with wild-type tomato. New Phytol. 161, 485–494.
453 Cordell, D., Drangert, J., White, S., 2009. The story of phosphorus: Global food
454 security and food for thought. Global Environ. Change 19, 292-305.
455 Cui, M., Caldwell, M., 1997. A large ephemeral release of nitrogen upon wetting of
456 dry soil and corresponding root responses in the field. Plant Soil 191, 291–299.
457 Drenovsky, R.E., Steenwerth, K.L., Jackson, L.E., Scow, K.M., 2010. Land use and
458 climatic factors structure regional patterns in soil microbial communities. Global
459 Ecol. Biogeog. 19, 27-39.

460 Facelli, E., Facelli, J.M., Smith, S.E., McLaughlin, M.J., 1999. Interactive effects of
461 arbuscular mycorrhizal symbiosis, intraspecific competition and resource availability
462 on *Trifolium subterraneum* cv. Mt. Barker. New Phytol. 141, 535–547.
463 Forster, J.C., 1995. Soil nitrogen., In: Alef, K., Nannipiero, P. (Eds.), Methods in
464 Applied Soil Microbiology and Biochemistry. Academic Press San Diego, CA., pp. 79–
465 87.
466 Franzluebbers, K., Weaver, R.W., Juo, A.S.R., Franzluebbers, A.J., 1994. Carbon and
467 nutrogen mineralization form cowpea plants part decomposing in moist and in
468 releatedly dried and rewetted soil. Soil Biol. Biochem. 26, 1379-1387.
469 Giovannetti, M., Mosse, B., 1980. An evaluation of techniques for measuring
470 vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84, 489-500.
471 Giovannetti, M., Sbrana, C., Avio, L., 2002. Arbuscular mycorrhizal fungal mycelium:
472 from germlings to hyphal networks., In: Gianinazzi, S., Schüepp, H., Barea, J.M.,
473 Haselwandter, K. (Eds.), Mycorrhizal Technology in Tropical Agriculture. Birkhäuser
474 Basel, Basel, Switzerland, pp. 49-58.
475 IPCC, 2013. Climate Change 2013: The Physical Science Basis. Intergovernmental
476 Panel on Cliamte Change.
477 Jentsch, A., Kreyling, J., Beierkuhnlein, C., 2007. A new generation of climate-change
478 experiments: events, not trends. Frontiers Ecol. Evol. 5, 365-374.
479 Johnson, N.C., Graham, J.H., Smith, F.A., 1997. Functioning of mycorrhizal
480 associations along the mutualism–parasitism continuum. New Phytol. 135, 575–586.
481 Knapp, A., Smith, M., 2001. Variation among biomes in temporal dynamics of
482 aboveground primary production. Science 291, 481–484.
483 Lambers, H., Raven, J.A., Shaver, G.R., Smith, S.E., 2008. Plant nutrient-acquisition
484 strategies change with soil age. Trends Ecol. Evol. 23, 95-103.
485 Li, X., Zhu, Y.-G., Cavagnaro, T.R., Chen, M., Sun, J., Chen, X., Qiao, M., Zhu, X., 2008.
486 Do ammonia-oxidizing archaea respond to soil Cu contamination similarly
487 asammonia-oxidizing bacteria? Plant Soil 324, 209-217.
488 Lynch, J.P., 2007. Roots of the second green revolution. Aust. J. Bot. 55, 493-512.
489 Meisnera, A., De Deyn, G.B., de Boerb, W., van der Putten, W.H., 2013. Soil biotic
490 legacy effects of extreme weather events influence plant invasiveness. PNAS 110,
491 9835–9838.
492 Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric
493 method for simultaneous detection of nitrate and nitrite. Nitric Oxide 5, 62-71.
494 Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and
495 staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of
496 infection. Trans. Br. Mycol. Soc. 55, 158-&.
497 Rillig, M.C., 2004. Arbuscular mycorrhizae and terrestrial ecosystem processes. Ecol.
498 Lett. 7, 740–754.
499 Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis, 3rd ed. Academic Press, New
500 York.
501 Tanaka, Y., Yano, K., 2005. Nitrogen delivery to maize via mycorrhizal hyphae
502 depends on the form of N supplied. Plant Cell Environ. 28, 1247-1254.
503 Treseder, K.K., 2004. A meta-analysis of mycorrhizal responses to nitrogen,
504 phosphorus, and atmospheric CO_2 in field studies. New Phytol. 164, 347-355.
505 van der Heijden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel,
506 R., Boller, T., Wiemken, A., Sanders, I.R., 1998. Mycorrhizal fungal diversity

507 determines plant biodiversity, ecosystem variability and productivity. Nature 396,
508 69-72.
509 Vierheilig, H., Coughlan, A.P., Wyss, U., Piche, Y., 1998. Ink and vinegar, a simple
510 staining technique for arbuscular-mycorrhizal fungi. Appl. Environ. Microb. 64, 5004-
511 5007.
512 Waite Analytical Services, Urrbrae, South Australia.
513 Watts-Williams, S.J., Cavagnaro, T.R., 2014. Nutrient interactions and arbuscular
514 mycorrhizas: a meta-analysis of a mycorrhiza-defective mutant and wild-type tomato
515 genotype pair. Plant Soil 384, 79–92.
516 Watts-Williams, S.J., Cavagnaro, T.R., 2015. Using mycorrhiza-defective mutant
517 genotypes of non-legume plant species to study the formation and functioning of
518 arbuscular mycorrhiza: a review. Mycorrhiza 25, 5870597.
519 Xiang, S.-R., Doyle, A., Holder, P.A., Schimel, J.P., 2008. Drying and rewtting effects
520 on C and N mineralization and microbial activity in surface and subsurface California
521 grassland soils. Soil Biol. Biochem. 40, 2281-2289.
522 Yu, S., Ehrenfeld, J.G., 2009. The effects of changes in soil moisture on nitrogen
523 cycling in acid wetland types of the New Jersey Pinelands (USA). Soil Biol. Biochem.
524 41, 2394–2405.
525
526
527

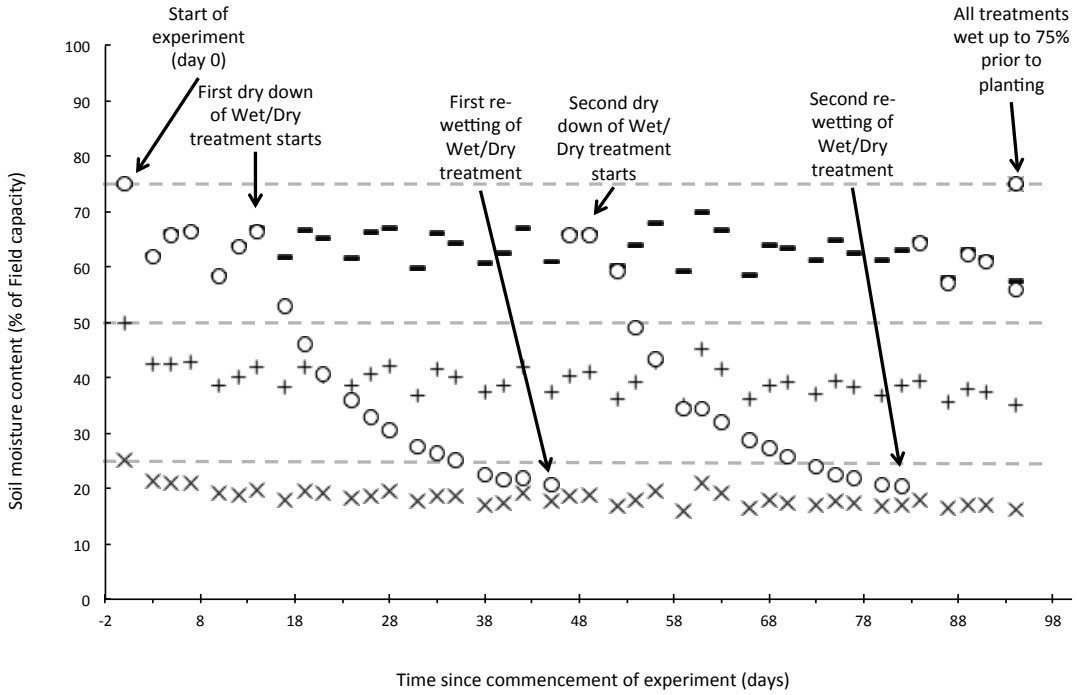


Figure 1. Soil moisture content (as % of Field capacity) of soils duing pre-planting period when legacy effects were established. Treatments: Dry (×), Wet (−), Intermediate (+),Wet/Dry (o). Values are Mean ± SE, n = 10. N.B. S.E. are very small and not visible on figure.

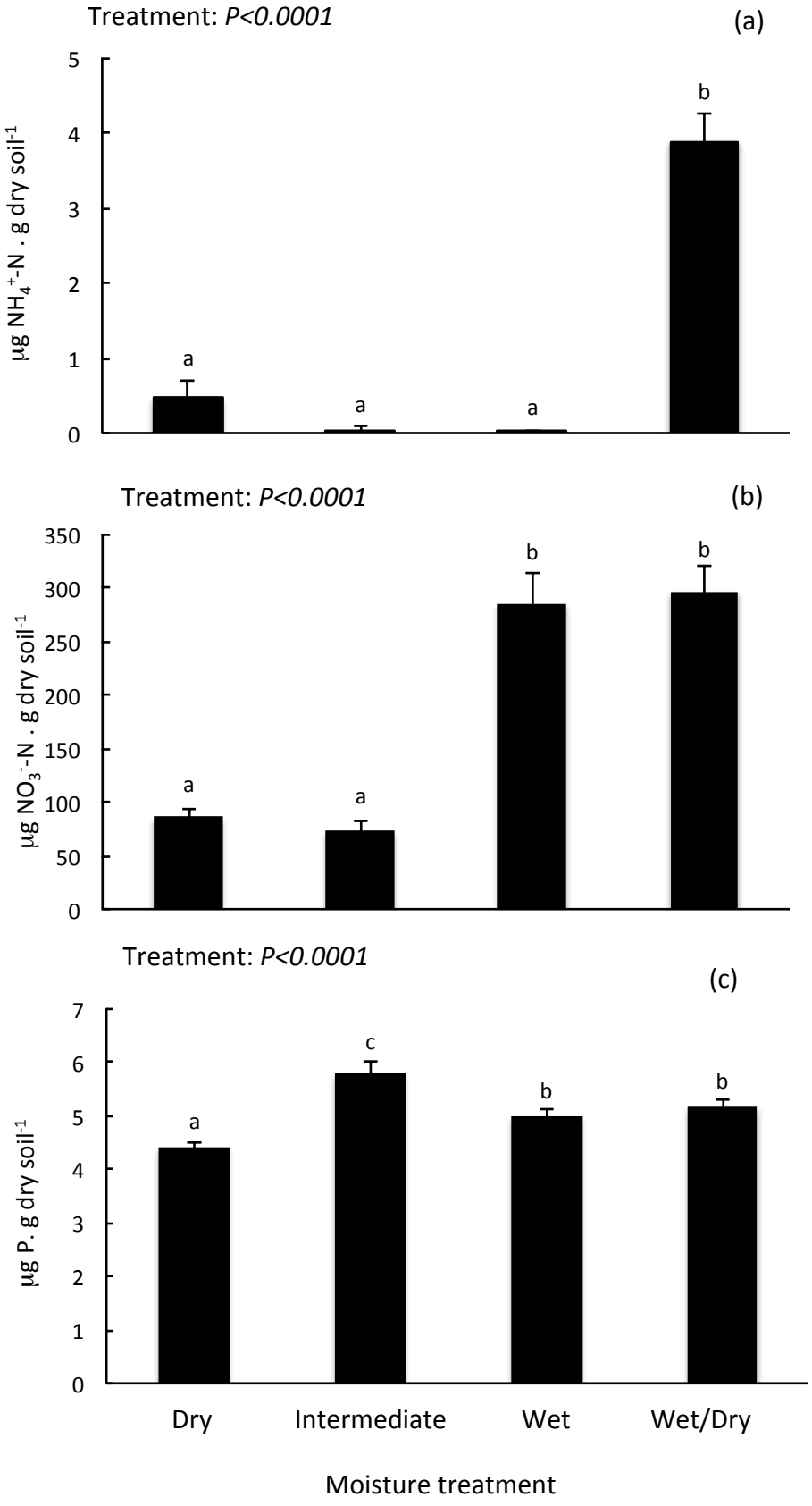


Figure 2. Soil (a) ammonium, (b) nitrate, and (c) plant-available P in soils at time of planting. Values are Mean ± SE, n = 8 – 10 (see text). Means followed by the same letter are not significantly different at the P<0.05 level (see text).

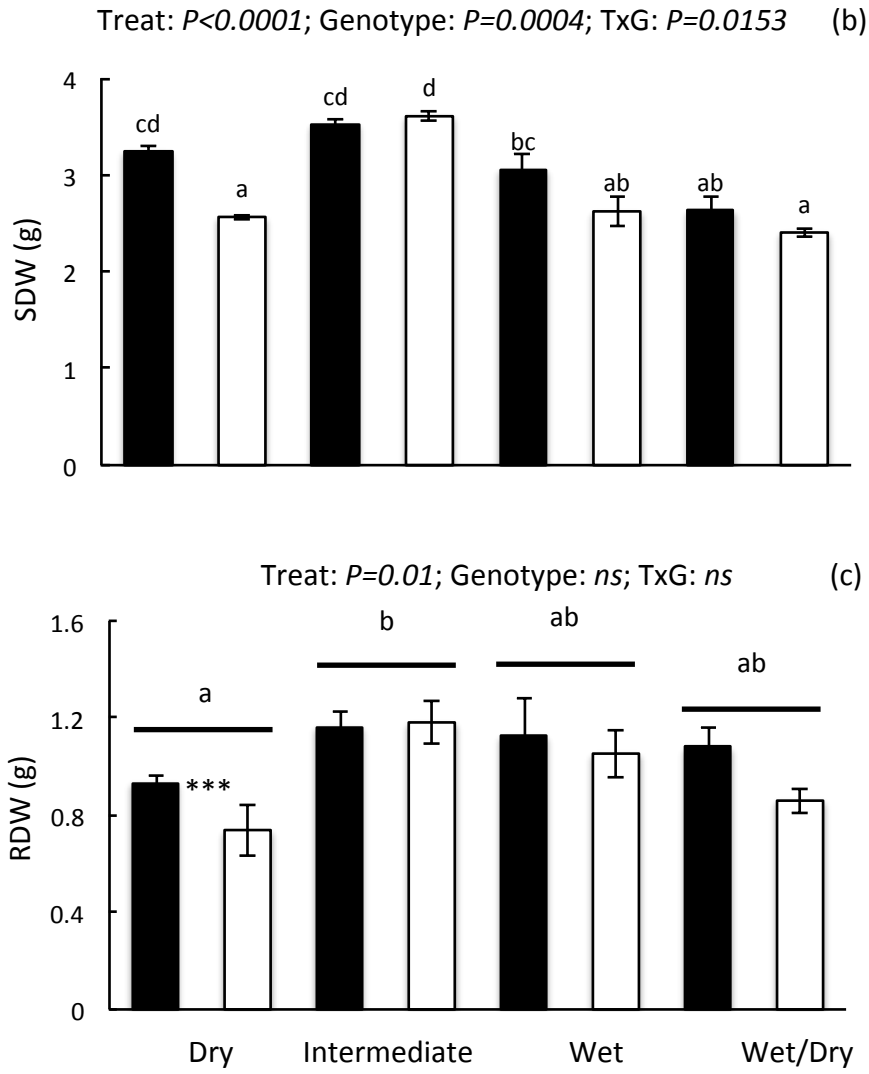
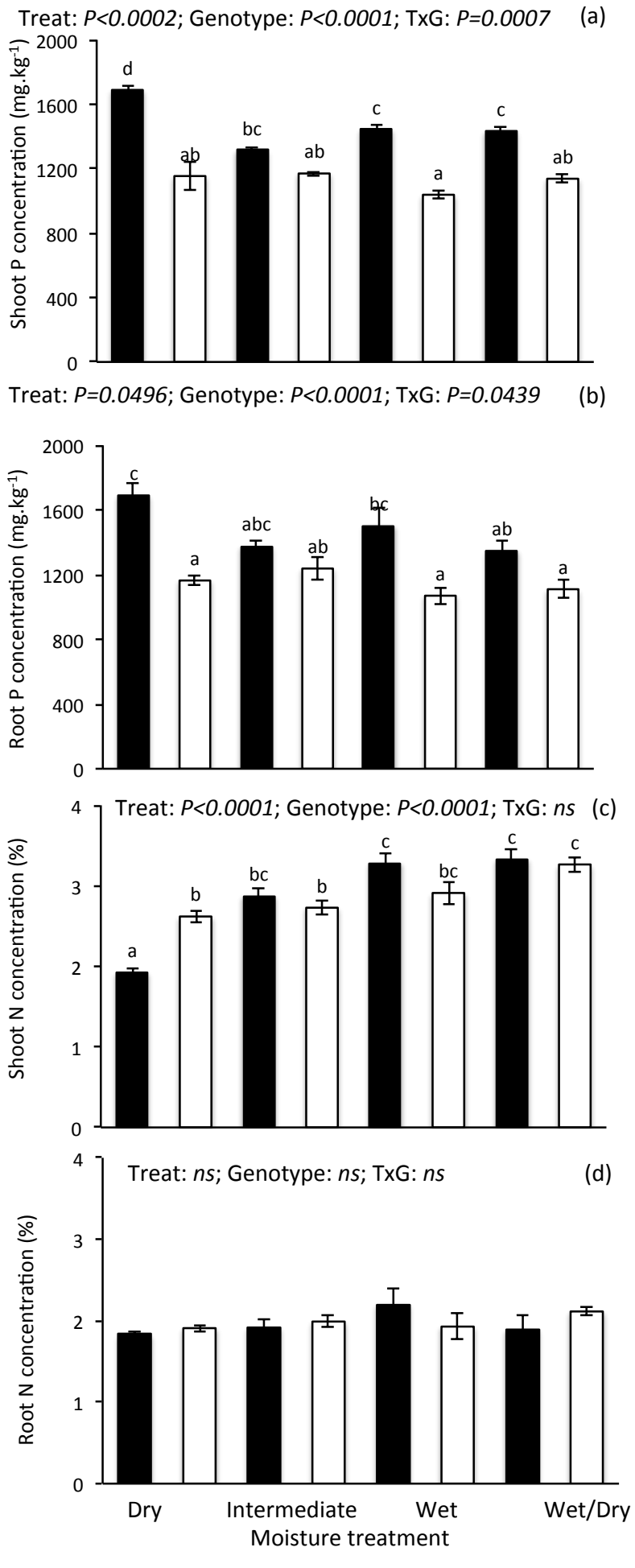


Figure 3 (a) mycorrhizal colonization of 76R plants, and (b) shoot dry weight and (c) root dry weight or 76R (black bars) and *rmc* (white bars) plants at the time of harvest. Values are Mean ± SE, n = 3 - 5 (see text). Means followed by the same letter are not significantly different at the P<0.05 level (see text).



Caption on next page

Figure 4 (a) shoot P concentration, (b) root P concentration, (c) shoot N concentration and (d) root N concentration of 76R (black bars) and *rmc* (white bars) plants at the time of harvest. Values are Mean ± SE, n = 3 - 5 (see text). Means followed by the same letter are not significantly different at the $P<0.05$ level (see text).