Root and arbuscular mycorrhizal effects on soil nutrient loss are modulated by soil texture.

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Abstract

Despite their importance, there is a lack of knowledge on the impact of forming arbuscular mycorrhizas (AM) on soil phosphorus (P) leaching in soils with different textures. Therefore, the objective of this study was to investigate the impacts of mycorrhizal and non-mycorrhizal roots on P leaching in two non-sterilised soils of contrasting texture. A mycorrhiza-defective tomato (*Solanum lycopersium* L.) genotype (named *rmc*), and its wild type progenitor that is able to form AM (named 76R), were used to investigate the effects of AM on P loss via leaching. Concentrations of reactive and un-reactive P in the leachate and soil were measured and related to plant growth, plant P uptake, soil water relations and leachate dissolved organic carbon (DOC) concentration. Soil texture affected mycorrhizal colonization, plant growth and plant P concentration, and influenced the concentration and chemical composition of P and the concentration of DOC leached. The chemical composition of P leached and P remaining in soil varied with soil texture, the presence or absence of roots and their arbuscular mycorrhizal status. Mycorrhizal plants reduced P lost via leaching in the sandy soil substrate, where DOC leached was also high. The roots, regardless of mycorrhizal colonization, appeared to have the greatest impact on increasing P and DOC leached. Taken together, this study provides new insights into the role of AM on soil P loss via leaching in soils of contrasting texture.

Introduction

Typically, less than 50% of soil-applied inorganic fertiliser is taken up by crops (Junguo et al. 2010). Nutrients not taken up by crops are prone to loss, for example, via leaching and surface run off, erosion or in gaseous forms (Junguo et al. 2010). When nutrients make their way into water bodies, water quality can be reduced (Boesch et al. 2001; Springmann et al. 2018), leading to eutrophication and biodiversity loss (Sharpley and Rekolainen 1997).

Arbuscular mycorrhizal fungi (AMF) are a group of near-ubiquitous soil fungi that can establish a symbiotic association with the roots of an estimated 80% of terrestrial plant species (Smith and Smith 2011). The potential for AM to reduce the risk of phosphorus (P) leaching in soil has been the subject of growing interest (Cavagnaro et al. 2015; Parihar et al. 2019). Various aspects of the impact of AM on soil P loss have been studied, including the importance of AMF species (Köhl and van der Heijden 2016), different host plant species (e.g. three different grassland species) (van der Heijden 2010), and different soil types (Bender et al. 2014). Experiments on the impacts of AM on soil nutrient loss have also been carried out using re-packed soil cores (Asghari and Cavagnaro 2012), intact soil cores (Asghari et al. 2005), field lysimeters (Bender and van der Heijden 2015), and nursery containers (Corkidi et al. 2011).

Although AM can reduce soil P loss via leaching, most studies have focused on analysing the total amount of P in the leachate, rather than the chemical nature of the P leached and/or remaining in the soil. Some insights, however, have been gained. For example, Bender et al. (2014) found that the formation of AM reduced the total amount of P and unreactive P leached. In contrast, in a previous study, we found an increase in both total and reactive P leached from soil with mycorrhizal plants, compared to non-mycorrhizal plants

67 (Tran et al. 2020). This highlights the need for further information on the impacts of roots and
68 AM on the leaching of P from soil in its various forms. Given the differences in the behaviour
69 of P in different forms in the environment (Toor et al. 2005), it is important to quantify not
70 only the total amount of P leached, but also its chemical nature (e.g. reactive and unreactive)
71 both in the leachate and the soil.

72 Although root and mycorrhizal assimilation of nutrients can help to reduce the loss of
73 nutrients via leaching, they can also modify the soil environment in ways that increase the
74 risk of nutrient loss. For example, root exudates (e.g. low molecular weight organic acids)
75 (Jaitz et al. 2011) can modify the rhizosphere and stimulate microbial activity (Nannipieri et
76 al. 2008), thereby affecting N (Brzostek et al. 2013) and P (Neumann G 2007) cycling and
77 availability, and thus, their propensity for loss via leaching. Similarly, carbon-rich root
78 exudates can increase soil dissolved organic carbon (DOC), which can directly or indirectly
79 bind with other soil nutrients (Nowack et al. 2008; Houben and Sonnet 2012). To this end, we
80 recently demonstrated that DOC in leachate was positively correlated with P leached (Tran et
81 al. 2020).

82 Soil P loss via leaching is complex and is affected by many edaphic factors, including
83 chemical, hydrological (soil permeability, soil aggregation) (Maguire and Sims 2002), and P-
84 sorption properties (Djodjic et al. 2004). Leaching of P is particularly problematic in sandy
85 soils where low P sorption capacity and relatively high hydraulic conductivity (Sims et al. 1998;
86 Nelson et al. 2005) can lead to significant P loss during rainfall events. Despite this, to our
87 knowledge very few studies focus on the effect of AM on P leaching in sandy soil. Moreover,
88 in our previous leaching experiment, the mean total P leached only accounted for 0.75 % of
89 P applied to the soil, and 0.44 % of the total P contents of the soil (i.e., applied P + existing

soil P) (Tran et al. 2020). This was likely due to the soil used (a loam containing of 62.9% clay and silt) having a high P absorption capacity. While previous work has focused on P leached from the soil, the studies of roots and AM on the amount and nature of P remaining in the soil are relatively few in number. To further explore this issue, there is a need to investigate impacts of roots and AM on soil P leaching in soils with varying textures.

Here we present results of a study in which we compare the impact of roots and AM on plant biomass, plant P uptake, composition of P forms (total P, reactive P and unreactive P leached) and DOC concentration in the leachate and soil P availability of two soil substrates. Specifically, we hypothesised that:

i. Roots and root colonization by AMF would affect soil moisture content and P mobilization and thus affect the leachate volume, the amount and composition of P in leachates and soils;

ii. The presence of plants would increase the P and DOC leached compared to no-plant treatments, regardless of soil texture; and

iii. A sandy soil substrate with lower clay content and water holding capacity would have less root colonization by AMF and thus more P and DOC leached compared to a soil with a higher clay content.

Materials and Methods

Microcosm systems

The microcosms used in this leaching experiment were constructed with PVC pipe (9 cm diameter × 35 cm height), following (Bowles et al. 2017). These pipes were fitted with a cap on the base that had a 15 mm diameter drainage hole, to which a PVC drainage outlet (15 mm diameter × 35 mm long) was fitted to allow collection of leachates. The PVC pipes were cut into three layers (0-10 cm, 10-25 cm and 25-35 cm) and then were carefully re-sealed using waterproof tape (T-rex 48 mm x 1.5 m 'ferociously strong tape', T-rex, USA), with a further layer of duct tape. This approach made it possible to cut the soil cores into three layers at the time of harvest (i.e. after leaching, see below). Filter paper was placed in the base of each microcosm to avoid soil loss, above which a 200 g layer of washed sand was placed to aid drainage.

The experiment was established with two ratios of sand:soil, two tomato genotypes (see below) and a plant free treatment; there were five biological replicates per treatment, giving 30 microcosms in total.

Soil, inoculum and nutrient addition

The soil used in this experiment was a fine sandy loam (25.71% clay; 37.19 % silt; 37.11 % sand) (Urrbrae red-brown earth (Alfisol)) collected from the 0-10 cm layer of the University of Adelaide's Waite Campus Arboretum, South Australia. The soil was air-dried and sieved to <2 mm to eliminate any coarse debris, and then mixed with fine sand (0.1-0.25 mm) at two different ratios: 70:30 and 10:90 (soil/sand, *w/w*); these are referred to as 'fine substrate' and 'coarse substrate', respectively, hereafter. The plant-available (Colwell) P of the fine substrate

and coarse substrates were 12 ± 0.5 and 5.5 ± 0.5 mg P kg^{-1} dry soil, respectively. The total P concentration in these substrates was 200 ± 4 and 104 ± 4 mg P kg^{-1} dry soil, respectively. The field capacity of the soil substrates was determined using a sintered glass funnel connected to a 1 m water column (Ψ_m = −10 kPa) (Cavagnaro 2016). Soil was packed in the glass funnel to the same bulk density as the collected field site (1.36 g/cm^3), saturated with RO water and allowed to drain for 48 h and then weighed. The soil was then dried at 105 °C for 48 h and soil gravimetric moisture content calculated. The gravimetric moisture content at field capacity of the fine and coarse substrates were 0.22 and 0.04 g water $^{-1}$ dry soil, respectively. Two kilograms of substrate was mixed with 100 g of AMF inoculum, amended with P (see below), then added to fill each microcosm.

The AMF inoculum used was *Rhizophagus irregularis* WFVAM10 (formerly named *Glomus intraradices*). The AMF had been previously cultured on *Trifolium subterraneum* L. (clover) cv. Mt Barker in 1 L pots containing soil: sand mix (10:90 *w/w*) for four months. The inoculum consisted of AMF spores, external hyphae and colonised root fragments (80-100% colonised by AMF) of the host plant in the dry substrate.

Each microcosm received 40 mg P, which is equivalent to 20 mg kg^{-1} dry soil, using $K_2HPO_4.3H_2O$ dissolved in 50 mL of reverse osmosis (RO) water, mixed thoroughly through the soil. This addition of P to the soils allowed sufficient mycorrhizal colonization and plant biomass in a preliminary experiment (data not shown). The final plant-available (Colwell) P concentration immediately following P addition was 30 ± 0.5 in the fine substrate and 19 ± 0.5 mg P kg^{-1} dry soil in the coarse substrate.

Non-mycorrhizal control and mycorrhizal plant treatments were established using a mycorrhiza-defective tomato (*Solanum lycopersicum* L.) mutant with reduced mycorrhizal

158 colonization (named *rmc* hereafter), and its mycorrhizal wild type progenitor (named 76R
159 hereafter) (Barker et al. 1998). This approach avoids the need to sterilise soil and thus ensures
160 a natural soil microbiome is present in the non-mycorrhizal treatment (Rillig et al. 2008).

161 Seeds of the 76R and *rmc* tomato genotypes were shaken in a 10% sodium
162 hypochlorite solution for three minutes to surface-sterilise the seeds. The seeds were then
163 rinsed with RO water, and sown into coarse sand for germination. The seedlings with fully
164 expanded cotyledons were transplanted into the microcosms (one seedling per microcosm)
165 after one week.

166

167 *Growth conditions*

168 Plants were grown in a glasshouse on The University of Adelaide's Waite Campus (Adelaide,
169 South Australia, Australia) from May to July 2019. Plants received 14.5/9.5-hour day/night
170 cycle supplemental lighting. The climate conditions in the glasshouse ranged from 15.6 - 23.7
171 °C, and 42.4 - 68.8 % humidity.

172 The microcosms were watered with RO water to 75 % of the water-holding capacity
173 (by weight) to avoid water being prematurely leached from the microcosms but still providing
174 sufficient water for plant growth. Plants were watered three times weekly, and were fertilised
175 with 30 mL of a modified Long-Ashton nutrient solution without P (Cavagnaro et al. 2001) in
176 the first week and then 10 mL weekly, thereafter. Also, 20 mg N as NH_4NO_3 solution (in RO
177 water) was added to all microcosms at 30 days after planting, following the appearance of
178 foliar symptoms of N deficiency.

179

Harvesting and leaching analysis

All plants were destructively harvested 56 days after planting. In order to eliminate water loss via transpiration during the leaching event, the shoots were cut at the soil surface. Aliquots of 200 mL of RO water were immediately added to the soil surface to initiate the leaching process. A total of 700 mL of RO water was added to the microcosms, simulating a rainfall event of 110 mm (Asghari and Cavagnaro 2012). After 48 hours, there was water remaining on the soil surface of the planted treatment pots, but leaching through the soil column had ceased.

Total P and molybdate-blue reactive P were measured on leachate passed through a 0.45 µm filter (unfiltered leachate was quite dark with particulate material). Total P in leachates was measured using inductively coupled plasma-optical emission spectrometry ICP-OES (Avio 200, Perkin Elmer). Molybdate-blue reactive P was measured colorimetrically (Murphy and Riley 1962) using a Multiskan Go (Thermo Scientific) plate reader. The difference between total P and (molybdate-blue) reactive P was calculated and is referred to as "un-reactive P" hereafter, following the terminology of Bender et al. (2014); (Toor et al. 2005). The concentration of dissolved organic carbon (DOC) in leachates was measured directly (non-filtered leachate) using a total organic carbon and total nitrogen analyser (Shimadzu).

Plant biomass and soil analysis

The soil microcosms were immediately separated into three layers at the previously cut and re-sealed points (0-10 cm, 10-25 cm and 25-35 cm) after the leaching event; the soil mass of the three layers was recorded. Approximately 100 g of soil was sampled from each soil layer for determination of the gravimetric water content, plant-available (Colwell) P, and total P. A

subsample of soil was dried at 105 °C for 24 hours to determine the gravimetric water content. The remaining soils for P pool analysis were dried at 40 °C in the oven for 24 hours.

The concentration of plant-available (Colwell) P in soil samples was determined using colorimetric assay (Murphy and Riley 1962). The soil samples were extracted with 0.5 M sodium bicarbonate ($NaHCO_3$) solution at a soil:extractant ratio of 1:100 following 16 hours shaking, according to a modification of Colwell (1963). The concentration of total P in soil samples was determined using an Avio 200 ICP-OES (Perkin Elmer), following heat block digestion with concentrated nitric acid and hydrochloric acid (Wheal et al. 2011).

The roots were collected from each soil layer by washing with RO water, and fresh root mass determined. A subsample (of known weight) of plant roots was stored in ethanol and then cleared with 10 % potassium hydroxide (*w/v*) at room temperature. After seven days, the cleared roots were rinsed and then stained in 5 % ink in vinegar solution at 60 ^{o}C for ten minutes (Vierheilig et al. 1998). The root length colonised by AMF was then determined on the stained root samples using the gridline intersect method for at least 100 intersections per sample (Giovannetti and Mosse 1980). The remaining roots and shoots were dried at 60° C for 48 hours, before root dry weight (RDW) and shoot dry weight (SDW) was determined. Dried plant material was ground to a fine powder and then digested with concentrated nitric acid and hydrogen peroxide using a heat block (Wheal et al. 2011). The concentration of P in shoots and roots was determined using ICP-OES (Avio 200, Perkin Elmer).

Statistical analysis

All statistical analysis was performed using R statistical software, Version 3.5.1 (R Core Team, 2019). Data were checked for the assumption of normality by analysing model residuals using a QQ plot and Shapiro-Wilk test. Two-way analysis of variance (ANOVA) was performed with *Soil substrate* treatment and *Plant* treatment (i.e. mycorrhizal plant, non-mycorrhizal plant, or no-plant), as factors in the analysis. Three-way ANOVA was performed on RDW, soil moisture and soil P with *Soil substrate*, *Plant* and *Soil depth* as factors in the analysis. In case of a significant interaction, means were compared using Tukey's HSD tests (at $\alpha < 0.05$).

Results

Mycorrhizal colonization, plant growth and nutrient uptake

Whereas roots of the *rmc* genotype were not colonized by AMF, those of the 76R plants in all treatments and each of the three soil layers, were (Figure 1). Specifically, roots of the 76R plants grown in the coarse soil, had a higher percent root length colonised in the lower soil layers than in the surface. In the fine substrate, colonisation was generally (albeit not significantly) lower than that of the coarse substrate, with no significant difference among soil layers.

The formation of AM had no impact on the plant biomass as there was no difference between *rmc* and 76R in terms of SDW or RDW (Figure 2a). While there was no difference in the RDW between the two soil substrates, there was a significantly higher SDW in the fine substrate compared to the coarse substrate ($P<0.001$).

There was no difference in root density between mycorrhizal and non-mycorrhizal roots between the three soil layers or two soil substrates (Figure 2c). The top layer (0-10 cm) had the highest root biomass in both soil substrates. The roots in the sandier soil mix had a higher density in the topsoil (0-10 cm) but lower in the bottom layer (25-35 cm) in comparison with roots in the fine substrate ($P<0.001$).

Whereas there was no difference in tissue P content between the *rmc* and 76R plants in the two soil substrates, the shoot P and root P content of plants in the coarse substrate were higher than those of plants in the fine substrate, irrespective of mycorrhizal status ($P<0.01$) (Figure 2b).

Leachate volume and nutrient content

After 48 hours, while all water added to the no-plant treatments had completely infiltrated the soil in the microcosms, there was water remaining on the soil surface of the treatments containing plants. The volume of water remaining on the surface of the microcosms containing mycorrhizal plants was 188 ± 6 mL and 97 ± 10 mL in the fine substrate and coarse substrate, respectively. The volume of water remaining on the surface of the microcosms containing non-mycorrhizal plants was quite similar with 160 ± 14 mL and 150 ± 20 mL remaining on the surface of microcosms containing the fine and coarse substrates, respectively.

In general, leachate volume was similar for the two soil substrates. There was no significant difference in leachate volume between mycorrhizal plants and non-mycorrhizal plants, but leachate volume was significantly lower in the presence of plants for both soil substrates (Figure 3). Additionally, whereas there was no difference in the leachate volume of the no-plant treatments between two soil substrates, within the plant treatments the coarse substrate had a significantly higher leachate volume than the fine substrate.

Reactive P accounted for a large proportion of P in all the leachate samples, comprising 80.6 ± 1.8 % of the P leached in the fine substrate and 64.1 ± 6.7 % of the P leached in the coarse substrate. In the absence of a plant, P concentration in the leachate for the coarse substrate was higher in the leachate of the fine substrate (Figure 4a). In addition, concentrations of total P and reactive P in leachates from the plant treatments were higher than those of the no-plant treatment (the only exception being the reactive P in the 76R plant of the coarse substrate). Furthermore, the unreactive P concentration in the leachate from the coarse substrate was higher than that from the fine substrate ($P<0.01$) (Table 1).

291 Specifically, the impact of AM on the concentrations of P leached was different between two
292 soil substrates; although there was no difference in the concentrations of leached P pools
293 (total P, unreactive P and unreactive P) between mycorrhizal and non-mycorrhizal plants from
294 the fine substrate, concentrations of total P and reactive P in leachates from the coarse
295 substrate were lower for mycorrhizal than the non-mycorrhizal treatments.

296 The DOC concentration of plant-free treatments was lower than for either the
297 mycorrhizal or non-mycorrhizal treatments, irrespective of soil substrate texture (Figure 4b).
298 The leachate from the coarse substrate had a higher DOC concentration than that from the
299 fine substrate ($P<0.001$) for all treatments. While AM did not influence DOC concentration in
300 leachates from the fine substrate, it increased the concentration of DOC in leachates from
301 the coarse substrate ($P<0.001$) (Table 1).

302

303 *Soil moisture and soil P*

304 The presence of plants reduced the post-leaching gravimetric water content of the soils in
305 fine substrate and slightly increased that of coarse substrate (Figure 5). The bottom layer (25-
306 35 cm) had the greatest water content, followed by the 10-25 cm layer at and the 0-10 cm
307 layer.

308 In general, unreactive soil P accounted for 70-98 % of the total soil P. Total P and
309 unreactive soil P concentration of the fine substrate was higher than that of coarse substrate
310 ($P<0.001$) (Table 2). There was no significant difference in the total and unreactive soil P
311 concentrations in term of soil depth and plant treatments (Figure 6).

312 Similar to the total soil P concentration, reactive soil P concentration of the fine
313 substrate was higher than that of the coarse substrate, especially in the upper two layers (0-

10 cm and 10-25 cm) of the fine substrate ($P<0.001$) (Table 2). While there was no significant difference in the reactive soil P concentration among three soil layers in the coarse substrate, the reactive soil P concentrations of the top and middle layers were higher than those of the bottom layer in the fine substrate. The presence of roots reduced the reactive soil P concentrations in the two first layers in comparison with the no-plant treatments. The absence of a plant resulted in greater reactive soil P concentrations for the plant treatments ($P<0.001$). In contrast, AM did not influence the concentrations of total soil P, reactive soil P, or unreactive soil P, after the leaching event.

Discussion

There was a strong effect of soil texture on plant growth, plant P concentration, formation of arbuscular mycorrhizas, leachate volume, leachate P and DOC concentrations, and the amount of P remaining in the soil after leaching. Whereas the presence of plants reduced leachate volume, the concentration of P and DOC in the leachates increased. Taken together, these results highlight the complex interactions between plants, AM and soil texture that work to modulate soil P loss via leaching.

The mycorrhizal status of plants had a significant impact on the amount, and chemical nature (reactive or unreactive), of P leached from the soil; this is consistent with previous studies (Köhl and van der Heijden 2016; Bender et al. 2014; Zhang et al. 2020). Here, however, the influence of AM differed between soils: whereas the formation of AM had no impact on P leached from the fine substrate, there was a significant reduction of total P and reactive P leached from microcosms with the coarse substrate in which mycorrhizal plants were grown. In previous studies where AM had no impact on P leaching, this was attributed to either a strong P-fixing ability of the soil used (Köhl and van der Heijden 2016), the absence of a positive mycorrhizal response (Duffková et al. 2019), or P leaching being negatively correlated with the colonization of extraradical mycorrhizal hyphae (Verbruggen et al. 2012). It is likely that all of these factors contributed to the results reported in the current study. For example, the coarse substrate is expected to have not only a higher hydraulic conductivity (see below), but also a lower P-fixing capacity, than the finer soil. Note that the lack of difference in the growth and P uptake of the mycorrhizal and non-mycorrhizal plants are consistent with the previous studies discussed above (Köhl and van der Heijden 2016; Duffková et al. 2019).

There is emerging evidence that plants and AM impact on P leaching, not only in terms of the amount of P leached, but especially the relative proportions of reactive and unreactive P (Bender et al. 2014; Tran et al. 2020). In the present study, we found that leaching of reactive and unreactive P, and plant/mycorrhizal effects on them, also differed with soil types. Specifically, mycorrhizal plants reduced the total P and reactive P leached from the coarse substrate but had no impact on P composition leached from the fine substrate. This suggests that the leaching of reactive P in a sandy soil substrate may be reduced in the presence of AMF. Importantly, reactive P fractions are not only a directly available P source for plants but also can comprise the majority of the leachate P from several soil ecosystems (Turner and Haygarth 2000; Heckrath et al. 1995; Toor et al. 2005). These results also provide new insights into the potential for AM to reduce different soil P fractions leached.

The reduction of P lost via leaching from the coarse substrate was due to a reduction in reactive P rather than unreactive P. In a previous study, the reduction of reactive P associated with AM was hypothesised to be due to the extension of mycorrhizal root systems compared to non-mycorrhizal roots enhancing P uptake from the soil (Bender et al. 2014; Jakobsen et al. 1992; Jansa et al. 2005). This cannot explain the reduction in our study as there was an absence of a greater plant growth or plant P uptake by the mycorrhizal plants. However, this reduction was associated with an increase in DOC leached from the mycorrhizal pots, and the presence of AMF has been previously shown to increase soil microbial biomass carbon (Xiao et al. 2019; Zarea et al. 2009). Thus, it may be that in the presence of AMF under high P availability in this substrate, soil microbial activity, and microbial P immobilisation, was stimulated; this is, however, speculative and is worthy of further investigation. Also, the increase in soil microbial activities might enhance the DOC production and leaching (Brooks et al. 1999; Christ and David 1996).

374 To our knowledge, this is the first microcosm study to determine P composition of the
375 soil after the leaching event. Unreactive P accounted for the majority of P in all soils, with the
376 reactive and unreactive P lower in the coarse substrate than the fine substrate. While soil
377 unreactive P concentration was the same among three soil layers, reactive P concentration
378 was lower in the bottom layer (25-35 cm) of fine substrate than two first layers. This might
379 be due to a greater water content in this layer resulting in more reactive P being released into
380 soil solution (Weaver et al. 1988) and leaching, thus leaving less reactive P remaining in the
381 soil. This highlights the impact of water movement through the soil core and how it may affect
382 the amount of P leaching loss (Djodjic et al. 2004). The presence of roots resulted in a lower
383 reactive P concentration in the top layers (coinciding with greater root density),
384 demonstrating the impact of roots and mycorrhizal roots on soil P.

385 A lower volume was leached from microcosms containing plants, with substantial
386 volumes of water retained on the soil surface after 48 hours. The presence of roots could
387 lower the infiltration rates and hydraulic conductivity compared to unplanted soil (Leung et
388 al. 2015) because roots have the capacity to block water flow channels created by soil pore
389 spaces (Buczko et al. 2007; Craig Scanlan 2010). Another possible explanation is that root
390 exudation might contribute to changes in the soil structure (Grayston et al. 1997; Traoré et
391 al. 2000) and thus soil pore size, which may reduce soil infiltration rate and hydraulic
392 conductivity. Although plant treatments had a lower leachate volume, concentration of DOC
393 and P in leachate of these treatments were consistently higher than for plant-free treatments
394 for both soil substrates. This can be explained by the contribution of root exudation (Nowack
395 et al. 2008; Boddy et al. 2007) and rhizosphere microbial activity (by using non-sterilised soil
396 substrate) (GoEdde et al. 1996) that would increase soil DOC. Also, DOC can interact with
397 many soil chemicals, affecting their fate in soil (Fernández-Pérez et al. 2005). The presence of

398 the DOC may decrease P absorption (Kang et al. 2011) because of the competition of organic
399 anions with P for sorption sites (Bhatti et al. 1998; Iyamuremye et al. 1996) or increase of
400 negative charge on soil surface that can inhibit P adsorption (Barrow 1989; Jiao et al. 2007).
401 The interaction of P with DOC has also been reported to increase the mobility of soil P (Zsolnay
402 and Görlitz 1994; Alvarez et al. 2004). Taken together, these results highlight that root and
403 AM impacts on soil P loss via leaching are more complex that a simple case of plant/AM P
404 assimilation.

405 Our use of a mycorrhiza-defective tomato mutant and its mycorrhizal wild-type
406 progenitor allowed us to investigate mycorrhizal effects on soil P leaching with the wider soil
407 biota intact (i.e. non-sterilised soil in all treatments) (Asghari and Cavagnaro 2012). Although
408 levels of AM colonisation were generally low, they were within the typical range for field
409 grown tomato plants (Cavagnaro et al. 2006; Bowles et al. 2016). Interestingly, colonisation
410 levels were higher in the roots of plants grown in the coarse substrate, and especially so, in
411 the lower soil layers. The higher levels of colonisation in the lower soil layers (coarse substrate
412 only), corresponded with lower root biomass. In addition, the greater level of mycorrhizal
413 colonization of roots in the coarse substrate was associated with greater P acquisition (both
414 in shoot and root) of plants grown in this substrate, compared to that of in fine substrate. The
415 higher levels of mycorrhizal colonisation of roots in the coarse substrate observed here is in
416 agreement with earlier work showing higher percent AMF colonization of roots grown in soils
417 with higher sand content (Zaller et al. 2011; Rodríguez-Echeverría and Freitas 2006).

418 In summary, the results of this study show the different effects of AM on P leaching
419 loss in two soil substrates differing in texture. This study also highlights the significant
420 contribution of soil texture on mycorrhizal colonization, plant growth, leachate volume and

soil P concentration and composition of the leachate. The presence of roots had a significant impact on leachate volume and the amount of nutrient leached. This finding shows that leaching of P from a plant-soil system is more complex than from a soil alone. The association of P with other soil nutrients (e.g. DOC), highlights the benefit of the non-sterilised soil approach (i.e. the mycorrhiza-defective mutant and its mycorrhizal wild-type progenitor) when evaluating soil nutrient loss because of the vital contribution of soil microbial community on nutrient cycling and leaching. It should be noted that the present study only included a single rainfall under greenhouse conditions; it will be important to investigate effects of AM on P and nutrient soil loss under field conditions with a 'natural' rainfall, or field irrigation, regime. It is also worth noting that AM impacts on the wider soil microbial community may have an impact on soil P cycling and DOC, and are also worthy of further investigation.

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663

- Impacts of roots and mycorrhizas on P loss from soils of contrasting texture were studied.
- P lost and remaining in soils varied with soil texture, the presence of roots and their mycorrhizal status.
- Mycorrhizal plants reduced P loss in the sandy substrate, associated with high DOC leached.

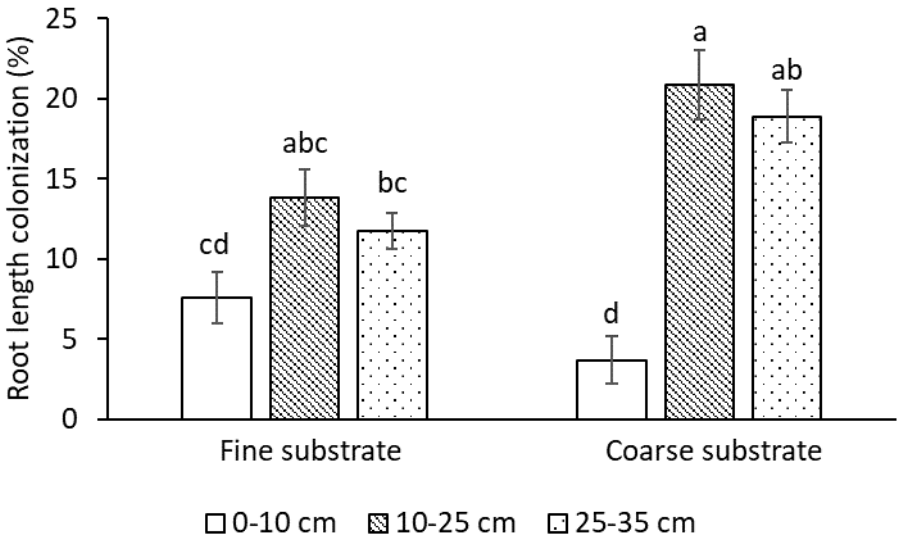

Figure 1. Root length colonization of mycorrhizal plants (76R). Values are mean ± SEM, n = 5. Means followed by the same letters are not significantly different (Tukey's HSD; α = 0.05)

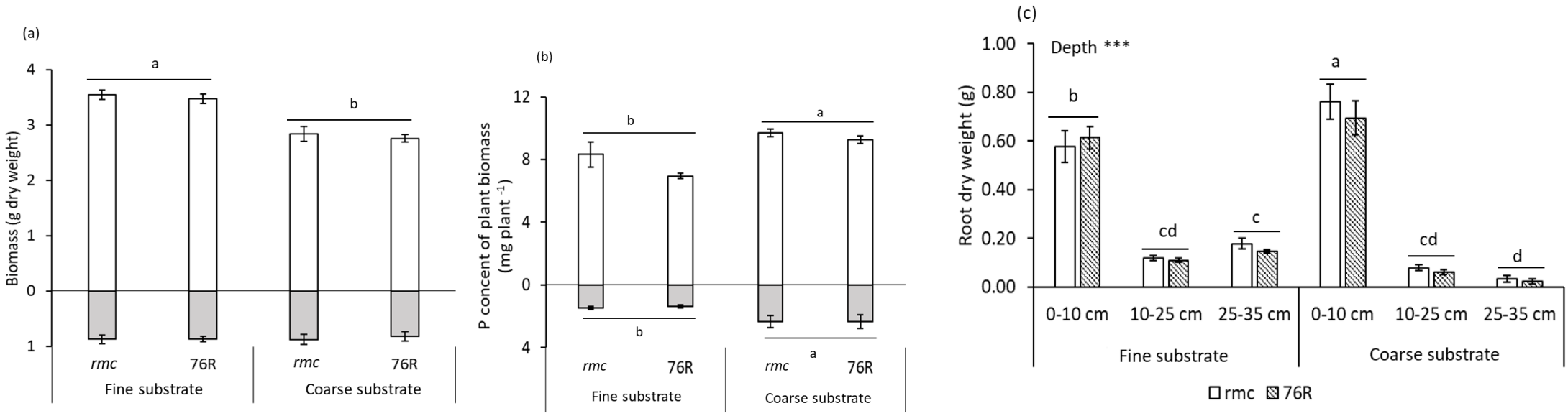

Figure 2. Mean shoot (above x-axis) and root (below x-axis) dry weight (a) and plant P content of the mycorrhizal plant (76R) and mycorrhiza-defective tomato genotypes (*rmc*) (b) and the root distribution at different soil depths and in two soil mixtures (c). Values are mean ± SEM, n = 5. Means followed by the same letters are not significantly different (Tukey's HSD; $\alpha = 0.05$).

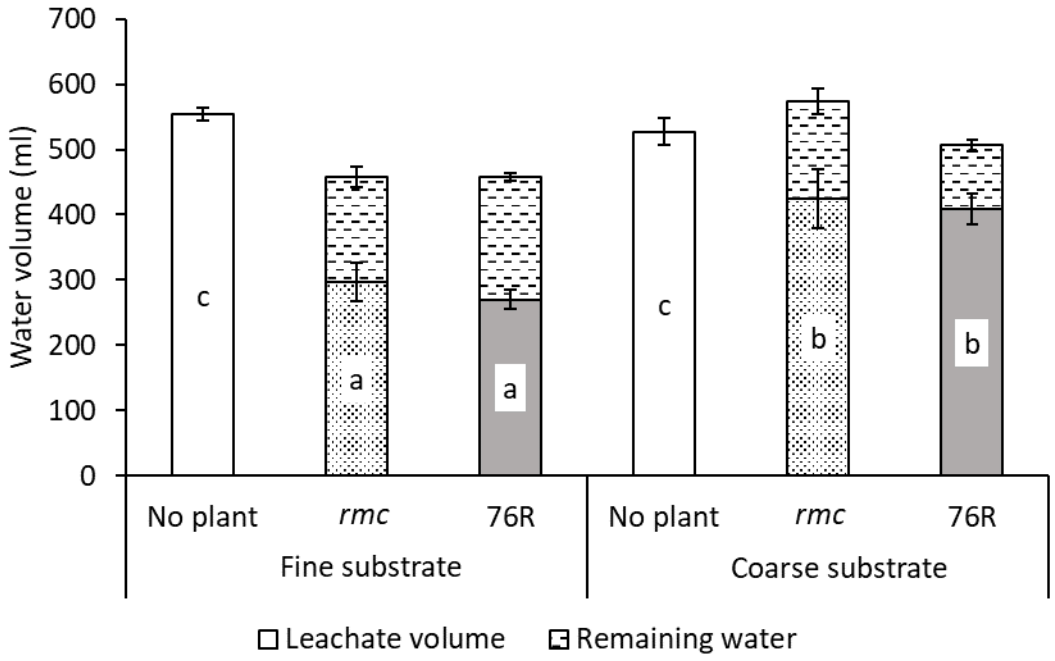

Figure 3. Leachate volume and water remaining on the soil surface after leaching event (mL). N.B. there was no water remaining on the soil surface at the end of the leaching event in the no-plant treatment. 76R and *rmc* are mycorrhizal and mycorrhiza-defective tomato genotypes; "No plant" refers to plant-free treatments. Values are mean ± SEM, n = 5. Means followed by the same letters are not significantly different (Tukey's HSD; $\alpha = 0.05$).

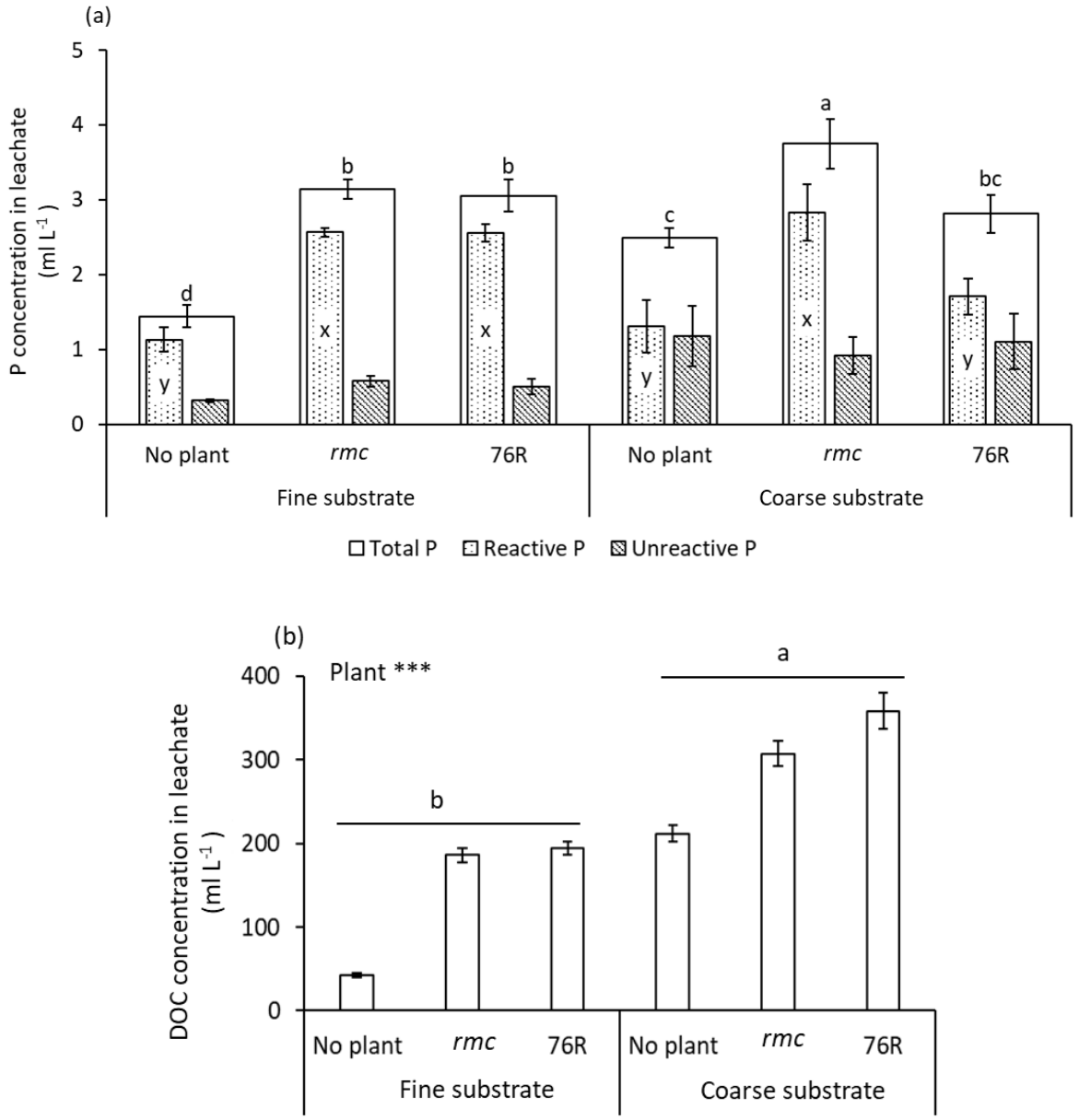

Figure 4. Phosphorus (a) and dissolved organic carbon (b) concentration of soil leachate. 76R and *rmc* are mycorrhizal and mycorrhiza-defective tomato genotypes; "No plant" refers to plant-free treatments. Values are mean ± SEM, n = 5. Means followed by the same letters are not significantly different (Tukey's HSD; $\alpha = 0.05$); "abcd" for total P and "xy" for reactive P.

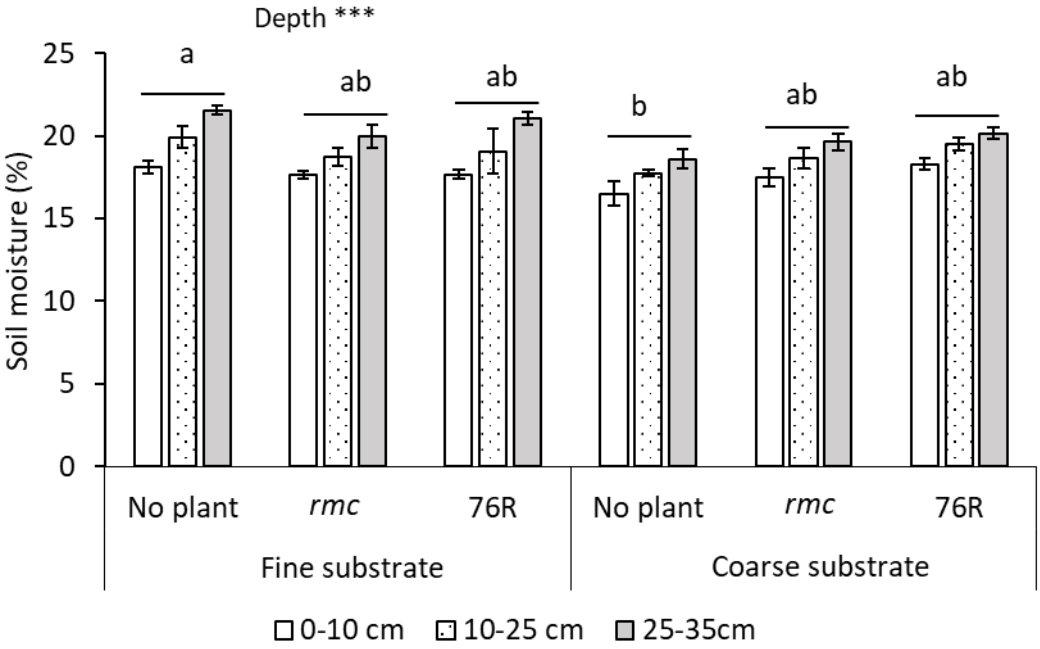

Figure 5. Gravimetric water content (%) of soils, following soil depth after leaching event. 76R and *rmc* are mycorrhizal and mycorrhiza-defective tomato genotypes; "No plant" refers to plant-free treatments. Values are mean ± SEM, n = 5. Means followed by the same letters are not significantly different (Tukey's HSD; $\alpha = 0.05$).

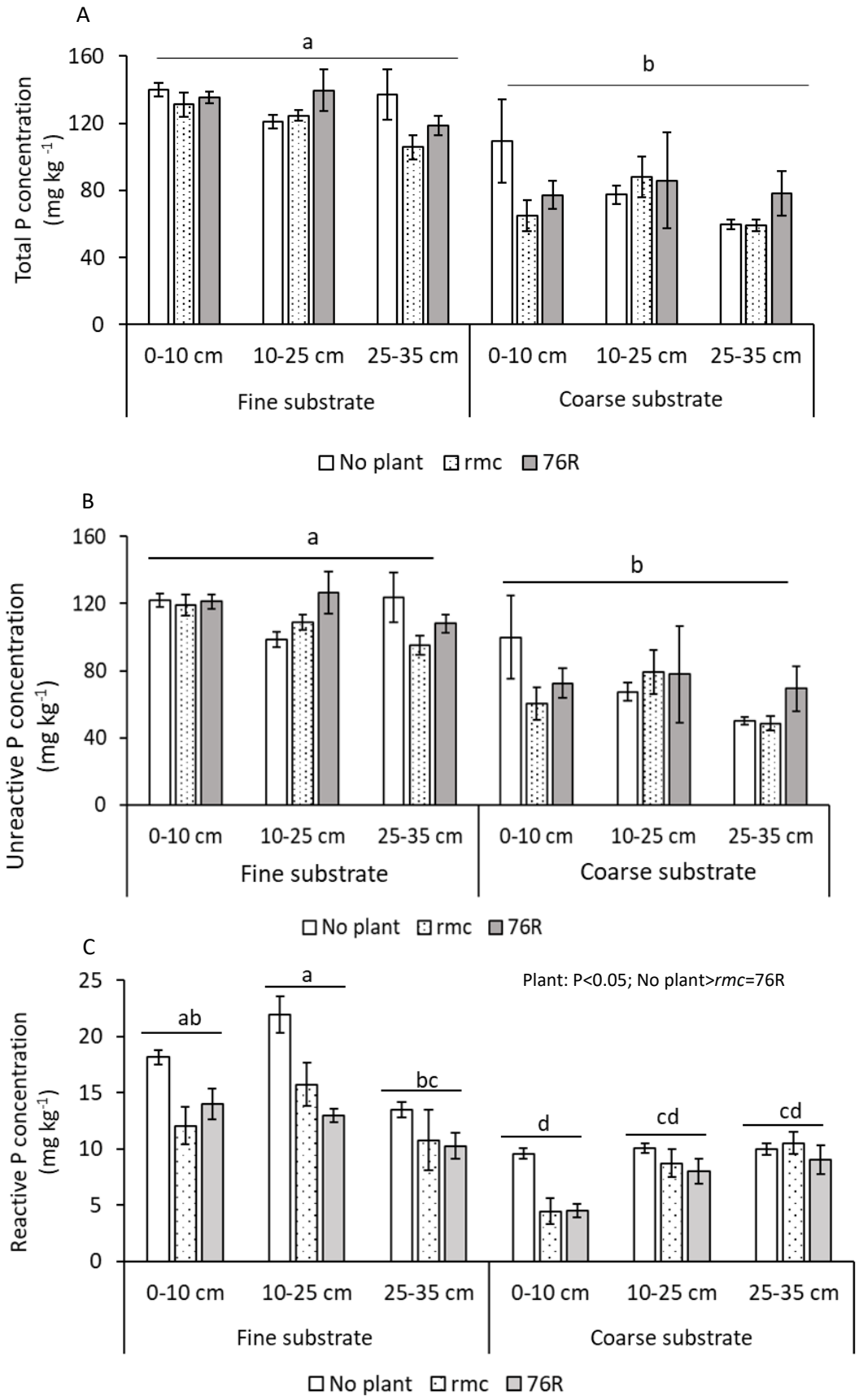

Figure 6. Phosphorus concentration (mg kg^{-1}) in soil samples, after the leaching event, following soil depth. (A) Total phosphorus, (B) reactive phosphorus, (C) unreactive phosphorus. 76R and *rmc* are mycorrhizal and mycorrhiza-defective tomato genotypes; "No plant" refers to plant-free treatments. Values are mean ± SEM, n = 5. Means followed by the same letters are not significantly different (Tukey's HSD; α = 0.05)

Supplementary

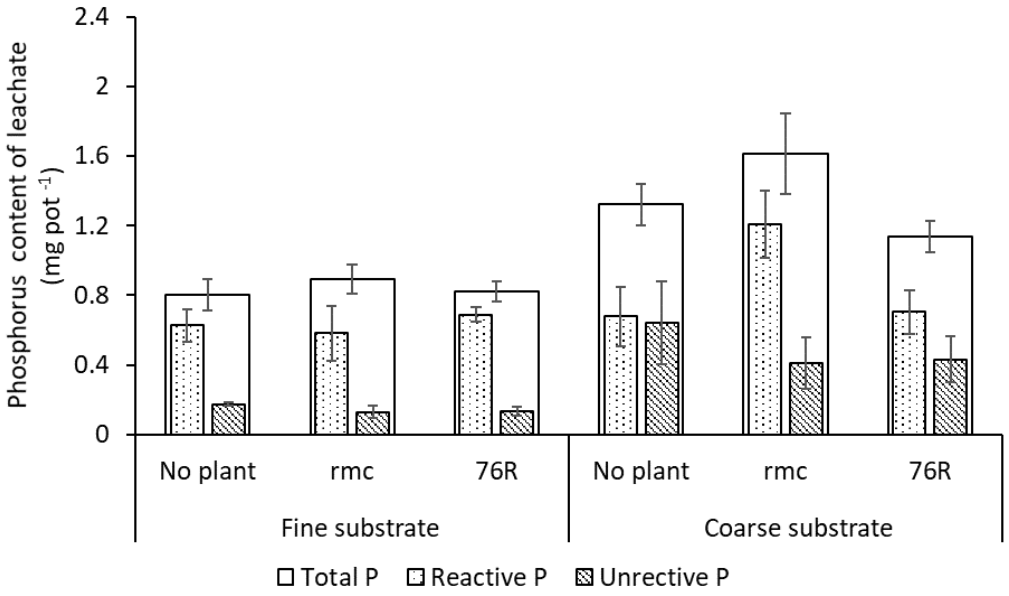

Figure S1. Phosphorus content of soil leachate. 76R and *rmc* are mycorrhizal and mycorrhiza-defective tomato genotypes; "No plant" refers to plant-free treatments. Values are mean ± SEM, n = 5.

Table 1. Two way ANOVA results for variables measured on plant and leachate. The plant factor of the plant variables had two levels (mycorrhizal plant and non-mycorrhizal plant), the plant factor of the leachate variable had three levels (mycorrhizal plant; non-mycorrhizal plant; and no -plant). “ns” indicates not significant; “*” indicates significant at p<0.05; “**” indicates significant at p<0.01; “***” indicates significant at p<0.001.

Variable	Soil substrate	Plant (*Mycorrhizal plant/non-mycorrhizal plant/No plant*)	Interaction
SDW	***	ns	ns
RDW (total)	ns	ns	ns
Shoot P content	***	ns	ns
Root P content	**	ns	ns
Leachate volume	***	***	**
DOC of leachate	***	***	ns
Leachate total P concentration	**	***	**
Leachate reactive P concentration	ns	***	*
Leachate unreactive P concentration	**	ns	ns
Leachate total P content	***	ns	ns
Leachate reactive P content	ns	*	ns
Leachate unreactive P content	**	ns	ns

Table 2. Three way ANOVA results for variables measured on root and soil; “ns” indicates not significant; “*” indicates significant at $p<0.05$; “**” indicates significant at $p<0.01$; “***” indicates significant at $p<0.001$.

	RDW (at each layer)	Soil moisture	Total soil P concentration	Unreactive soil P concentration	Reactive soil P concentration
Soil substrate	ns	**	***	***	***
Plant	ns	ns	ns	ns	***
Soil Depth	***	***	ns	ns	**
Soil substrate : Plant	ns	**	ns	ns	ns
Soil substrate : Soil depth	***	ns	ns	ns	***
Plant: Soil Depth	ns	ns	ns	ns	ns
Soil substrate : Plant : Soil Depth	ns	ns	ns	ns	ns