THE UNIVERSITY of ADELAIDE

THE EFFECTS OF ARBUSCULAR MYCORRHIZAS ON PHOSPHORUS CYCLING AND LEACHING IN SOILS

In partial fulfilment of the requirements for the degree of

Doctor of Philosophy

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Thesis abstract

The leaching of phosphorus (P) from soils is of major concern worldwide. In agricultural production systems P leaching represents an inefficient use of a limited resource, as well as potentially leading to contamination of aquatic systems. Arbuscular mycorrhizal fungi (AMF) establish a symbiotic with the majority of terrestrial plants. When the fungi form these associations with plants, they can take up P from the soil and deliver it to the plant. In doing so, they not only improve plant P nutrition, but can also reduce the risk of soil P loss via leaching. While effects of arbuscular mycorrhizas (AM) on plant P acquisition are very well understood, their impacts on soil P leaching are only just starting to be explored.

The aim of the work presented in this thesis was to study the effects of AM on soil P leaching, with an emphasis on P dynamics in the soil-plant-leachate system. A series of greenhouse and field experiments were conducted to explore this issue, including: two microcosm studies to investigate mycorrhizal and soil effects on plant biomass, plant P nutrition, soil P after leaching events, leachate volume, and the amount and chemical composition of P in leachates (Chapter 2 and 3); a field-based study to investigate mycorrhizal effects on fruit yield, soil moisture, soil (16S) bacterial community composition, and soil P loss under realistic field conditions (Chapter 4); and a final set of experiments using nuclear magnetic resonance (NMR) spectroscopy, to investigate the storage of P in the external hyphae of AMF (Chapter 5). All leaching experiments (field and glasshouse) made use of a mycorrhiza defective tomato mutant and its mycorrhizal wild-type progenitor, to study AM effects in the field. This approach avoids the potentially confounding effects of soil sterilisation, which is commonly used to establish non-mycorrhizal control treatments.

The outcomes of the work presented in this thesis confirm the positive impacts of AM on plant P uptake, plant biomass and tomato fruit yield and nutrients (Chapters 2, 3, 4 and 5). The presence of roots, regardless of mycorrhizal colonization, had a significant impact on soil P, total P and the chemical composition of P in leachate (Chapter 2 and 3). An important finding of this work was that roots increased the concentration of dissolved organic carbon (DOC) in leachate, and this increase in DOC concentration coincided with an increase in the concentration of P leached (Chapter 2 and 3). In the field experiment, AM had no significant effect on soil moisture, leachate volume or soil (16S) bacterial communities (Chapter 4). Importantly, soil texture affected mycorrhizal colonization, plant P and the amount of P and DOC leached, highlighting the need for results to be carefully considered in context (Chapter 3). This may be especially important in the context of nutrient leaching given the importance of soil texture in water

movement through soil. While not a major theme of the work presented here, the ^{31}P NMR spectra identified a polyphosphate (PolyP) peak in mycorrhizal external hyphae, confirming the importance of PolyP in hyphal P storage (Chapter 5). Taken together, this study provides new insights into the impacts of the below-ground plant systems and AMF on soil P leaching in soils.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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Cuc Tran

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List of publications

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Chapter 1 - Introduction

This thesis is presented as a series of published (Chapter 2 and 3) and as yet un-submitted (Chapter 4) journal manuscripts, and a standalone chapter (Chapter 5). Each of these papers/chapters begins with its own detailed introduction/ literature review. Accordingly, this introductory chapter contains a short literature review that seeks to provide context for the project, and to identify major knowledge gaps. This approach has been taken in the interests of avoiding repetition. This chapter concludes with a statement of aims and an outline of the thesis structure.

1.1 Literature review

1.1.1 Soil P leaching

Soil nutrient leaching is defined as the downward movement of dissolved nutrients in the soil profile with percolating water (Lehmann and Schroth 2003). Nutrient loss via leaching from agricultural ecosystems depletes soil fertility, accelerates soil acidification, and reduces plant productivity and sustainability (Wardle et al. 2004; Laird et al. 2010). Moreover, leached nutrients can enter the groundwater and lead to many environmental pollution issues, such as eutrophication of surface water, algal blooms and a reduction in aquatic biodiversity (Carstensen et al. 2014).

Nutrients that are readily leached from soil include phosphorus (P), nitrogen (N), potassium (K) and zinc (Zn) (Adesemoye and Kloepper 2009). Among these nutrients, soil P leaching has become a major concern because of the global depletion of high-quality mineral P resources used for chemical fertilizer production (Cordell et al. 2009b; Vance 2001). Soil P loss is especially problematic as small amounts of P leached from soil to aquatic systems can lead to eutrophication and biodiversity loss (Sharpley and Rekolainen 1997). In addition, P, the focus of the work presented in this thesis, is an essential plant macronutrient that in many soils is deficient (Roberts and Johnston 2015).

Many agricultural soils have a high P absorption capacity (Baker et al. 1975) as P is relatively immobile in most soils, forming insoluble complexes with calcium and manganese at high pH; and with aluminum and iron at low pH (Nye and Tinker 1977). As a result, P uptake from soil can be limited; it is estimated that only 10-30% of P fertilizer applied is taken up by plants (McLaughlin M J 1991) in the year of application. Therefore, the rates of P fertilizer applied to soils are often much higher than plant demand, so as to ensure that available P in soil solution is not limiting to crop production (Frossard et al. 2000). Although P leaching from soils has long been considered negligible in most settings (Brookes et al. 1997), it occurs in regions of intensive agriculture with high rates of P application, and is especially important in sandy soils where there is less opportunity for P to bind to soil particles, and water leaches more readily (Chen et al. 2006). Phosphorus loss from soils via leaching and surface run off can be substantial, and losses of up to 30 kg P her hectare annually have been reported in some areas (Herzog et al. 2008; Sims et al. 1998). Taken together, there is an urgent need to reduce nutrient leaching in soil systems as well as increase plant nutrient uptake efficiency in order to minimize P fertilizer

inputs, reduce 'waste' of P inputs, and avoid potentially harmful impacts of P loss from agricultural soils.

1.1.2 Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) are a widespread group of soil fungi that establish a symbiotic association with the roots of most plant species (Smith and Smith 2011). When AMF colonize roots, they form what are referred to as arbuscular mycorrhizas (AM). The formation of AM can enhance plant nutrient acquisition, reduce plant stress, and enhance plant resistance to pathogens (van der Heijden et al. 2003; Smith and Read 2008). Thus, AMF have an important role to play in achieving agricultural sustainability. While AMF are best known for their ability to take up P and deliver it to plants (Marschner and Dell 1994), they can also take up other nutrients including Zn (Cavagnaro 2008; Watts-Williams et al. 2017), N (Atul-Nayyar et al. 2009) and Cu (Clark and Zeto 2008). It is for this reason that AMF have been suggested as having an important role in potentially reducing soil P loss via leaching see Cavagnaro et al. (2015) for recent review. In exchange, AMF receive carbon from plants (Lehmann et al. 2014); AMF may receive up to 20% of recently-fixed carbon from the host plants (Soudzilovskaia et al. 2015; David et al. 2000). This supply of C to the AMF usually comes at the expense of plant root growth, which may in turn have consequences on plant root interception of P and water (and other nutrients) during leaching events.

The symbiosis between roots and AMF has been considered as a plant strategy to overcome soil P deficiency stress (Réka et al. 2009). The beneficial effects of AM on plant growth, especially the increase in plant P uptake, are very well-documented. Various mechanisms for this phenomenon have been reported including exploration of larger soil volume (Tinker 1978), faster movement of P into mycorrhizal hyphae (Bolan et al. 1987) and solubilization of soil P (Hetrick 1989). Javot et al. (2007) and Smith and Smith (2011) also revealed molecular and physiological evidence for the expression of P transporter genes of AMF and plants affected by the formation of AM. Mycorrhizal plants have also been reported to increase the uptake of poorly soluble P sources, such as iron and aluminum phosphate and rock phosphate (Bolan 1991). This may be important in the context of P leaching because AM can increase P uptake and thus reduce the amount of available soil P that otherwise would be at risk of being lost.

The uptake of P by AMF is strongly influenced by the spatial distribution of mycorrhizal hyphae in the soil (Jakobsen et al. 1992). The hyphae of AMF can extend beyond the root surface

by more than 10 cm (Li et al. 1991) and hyphal density per gram of soil is estimated at more than 10 metres (Jakobsen et al. 1992; Cavagnaro et al. 2005). Moreover, in comparison with root hairs, fungal hyphae can penetrate smaller soil pores due to their smaller diameter (Allen 2011). As a result, AMF-associated root systems can explore and exploit a greater volume of soils than non-mycorrhizal root systems (i.e., roots only) and thus enhance P plant uptake, even beyond the root nutrient depletion zone (Rouphael et al. 2015). This large network of hyphae may allow AMF to rapidly respond to, and intercept, pulses of nutrients as they move through the soil profile. Taken together, it is increasingly clear that AM have a role in reducing P leaching loss in soils.

1.1.3 Arbuscular mycorrhizas and soil P leaching

There is a growing body of evidence that suggests a role for AM in reducing soil nutrient loss via leaching (Asghari et al. 2005; Corkidi et al. 2011; Bender and van der Heijden 2015; Asghari and Cavagnaro 2011). A summary of this work, including experimental approach, soil type, plants and fungi, and major findings, is provided in Table 1. The effects of AM on soil nutrient leaching are mainly attributed to the capacity of fungal hyphae systems to enhance plant P uptake, even beyond rhizosphere depletion zones, and thus greater removal of soil available P (Cavagnaro et al. 2015). There are, however, contrasting results between studies. For example, Verbruggen et al. (2012) found that the amount of soil P leaching loss was negatively correlated with the abundance of fungal hyphae and mycorrhizal effects on soil P leaching loss were variable depending on soil inoculum used. Some studies have also shown that the AM-mediated reduction in soil P loss via leaching was important only when soil P levels were low (Asghari et al. 2005; van der Heijden 2010). This is consistent with much previous research demonstrating that AM formation is reduced when available soil P is relatively high (Gosling et al. 2013; Smith and Smith 2011). Importantly, a beneficial impact of AM on P leaching is not always reported. Some previous studies indicated no or little mycorrhizal effect on soil P leaching (Köhl and van der Heijden 2016) or an increase in P loss with AMF (Bender and van der Heijden 2015). Moreover, soil P loss via leaching is complex and affected by many soil biological, physical and chemical properties (Huang et al. 2011). Mycorrhizal effects on soil P leaching have been reported to be influenced by various factors, including strong soil P fixing capacity (Köhl and van der Heijden 2016); the mineralization and mobilization of soil P by soil microbes (Bender and van der Heijden 2015); different host plants (Corkidi et al. 2011) or colonizing AMF species (Köhl and van der Heijden 2016). Taken together, while it is apparent that AM can have an impact on soil P loss via leaching, results among studies are variable. It is in this context that this project

was devised. I will now present a number of research gaps that I have identified, and consider warrant investigation.

Table 1. Overview of studies investigating mycorrhizal effects on soil P loss via leaching

AMF species	Host plants	Experimental systems	Soil types	P loss response to AMF treatments	Explanation	References
Glomus intraradices	Clover	Microcosm greenhouse	Sterilized loamy sand	↓ P leached 2.7 times under low P conditions No difference where soil P high	AM ↑ plant growth and plant P removal	Asghari et al. (2005)
Commercial mycorrhizal inoculants (Seven AMF species)	Morning glory	Pots/outdoor in summer	Steam-pasteurized soil less substrate	AM had minimal effects on pH and EC of leachate	Greater nutrient utilization-potential ↓ risk of nutrient leached	(Carpio et al. 2005)
Glomus intraradices	Three grass species	Microcosm greenhouse	Autoclaved dune sand	↓ 60% P loss under low nutrient conditions No difference under nutrients rich conditions	AM promote a closed P cycle	(van der Heijden 2010)
Soil AMF (soil filtrate)	Grass	PVC column glasshouse	Autoclaved loam soil	↓1.4 times P concentration in leachate	↑ mycorrhizal root biomass and nutrient uptake	(Asghari and Cavagnaro 2011)
Glomus species (commercial product)	Sunflower and lemonade berry	Nursery container	Steam sterilized mixture of saw dust, clay and sand	↓ orthophosphate in leachate	↑ nutrient uptake	(Corkidi et al. 2011)

Glomus intraradices	Maize		soil sand mixture	Varied depending on soil inoculum used	P leached negatively correlated with AMF abundance	(Verbruggen et al. 2012)
Soil AMF	Red clover	Microcosm greenhouse	Autoclaved soil sand mixture	↑ 20% unreactive P No effects on dissolved organic P	↑ plant productivity	(Kohl et al. 2014)
AMF isolated from grassland soils	Grassland	Microcosm greenhouse	Sterilized soil sand mixture	↑reactive P and ↓ unreactive P leached	↑ uptake of P and mineralization of organic P compounds	(Bender et al. 2014)
Soil AMF	Crop rotation (maize/grass/ wheat/clover*)*	Outdoor lysimeter	Sterilized sand-soil mixture	↑ total P loss in Year 1 and no effects in Year 2	Enhanced mineralization and mobilization of soil P by soil microbes	(Bender and van der Heijden 2015)
Three AMF species	Grass and legume	Microcosm; greenhouse	Gamma-sterilized soil sand mixture	No effects	Strong P soil fixing ability	(Köhl and van der Heijden 2016)
Three AMF strains	Grassland	Microcosm; greenhouse	Autoclaved dune sand	↓ 50% P loss	AMF enhanced the soil nutrient interception ability	(Martinez-Garcia et al. 2017)
Rhizophagus irregularis	Maize	Pot; greenhouse	Sterilized arable soil	No effects	AM had no impacts on soil nutritional regimes	(Duffková et al. 2019)
Soil AMF	Rice pairs of mycorrhiza-defective	Microcosm; greenhouse	Soil sand mixture	↓ 11% P leaching	AMF ↓ P loss via runoff and leaching	(Zhang et al. 2020)

	mutant and its progenitor					
Soil based AMF	Maize	Dual-compartment system; greenhouse	Autoclaved soil	↓ 21-39% P leaching loss	AMF ↓ interflow P	(He et al. 2021)

1.1.4 Research gaps

Phosphorus is present in the soil in various chemical forms. These P-containing compounds/pools may differ in their propensity to be leached from the soil, due to differences in their behavior in the soil environment (Stutter 2015; McDowell et al. 2021). Phosphorus can be leached from soils in reactive forms which are directly available to plants (dissolved PO_4^3-P), and unreactive forms that are not (e.g. soluble and particulate organic P compounds, polyphosphates and particulate inorganic materials) (Pote et al. 2009). Previous work has shown that these fractions make up a significant part of total soil P lost by leaching (Bender et al. 2014). Unreactive P compounds can contribute 60% to 88% of total P leached (Ulen 1999; Neumann et al. 2012). If we are to better understand (and indeed minimize) P loss via leaching, it is important to study the different P forms and their behavior in the soil environment (Marc 2015). However, previous research has only focused on the impact of AMF and the quantity of P leached, whereas less attention has been paid to the chemical composition of the P leached, which is an important key to exploring this phenomenon in depth. Bender et al. (2014) observed a 31 % and 24 % reduction in total P and unreactive P in leachate (respectively), when plants formed AM (i.e. compared to non-AM control treatments). In contrast, an 20 % increase in the leaching of unreactive P in some mycorrhizal treatments has been reported (Kohl et al. 2014). Because of the potential contribution of various types of P compounds in the leachate and mycorrhizal effects on different P compounds may also be varied, it is necessary to identify the composition of all P leached under the AMF-plant systems to address the question of whether AMF can reduce overall P soil loss, or only a certain form of P. An understanding of this will be important to effectively manage the risk of P leaching in the environment. The issue of the chemical nature of pools of P leached is explored in Chapters 2 and 3.

AMF can improve soil structure (Rillig and Mummey 2006) and soil water retention (Augé 2004), thereby playing an indirect role in reducing leachate volume. However, the impacts of AM on leachate volume are not consistently reported in the literature (Asghari and Cavagnaro 2012; van der Heijden 2010). As root and AM effects on leachate volume may contribute to a reduction in P loss via leaching, this is a matter that requires further study. Arbuscular mycorrhizas can also improve plant water use efficiency (Bowles et al. 2016) and alter soil moisture dynamics, which can in turn influence other soil physical, chemical, and biological properties and processes. While mycorrhizal effects on the leachate volume and nutrient concentrations in leachates are well documented (van der Heijden 2010; Asghari and Cavagnaro 2011; Köhl and van der Heijden 2016; Corkidi et al. 2011; Bender and van der Heijden 2015;

Bender et al. 2014), their impact on soil moisture dynamics have not been studied in detail. Therefore, mycorrhizal effects on soil moisture and their association with soil P leaching under greenhouse and field conditions were included in this study (Chapter 2, 3 and 4).

Soil microbial communities (beyond AMF) play an important role in the cycling of P in agroecosystems (Dai et al. 2020). Soil microbial communities can the alter soil nutrient availability and thus nutrient cycling and leaching (Rillig 2004). Soil microorganisms can produce compounds that stimulate mycelial growth of AMF or enhance mycorrhizal formation (Barea et al. 2002). AMF can, in turn, alter soil microbial diversity (Rillig et al. 2006a; Marschner et al. 2001) and/or bacterial abundance (Nuccio et al. 2013), and/or specific functional bacterial groups such as phosphorus-solubilizing bacteria (Kim et al. 1997). Arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria have been found to interact, thereby increasing soil P solubilization and plant growth (El Maaloum et al. 2020; Nacoon et al. 2020). Despite this, the interaction between soil microbes and AMF or their impacts on soil P loss have not, to my knowledge, been investigated. In recent years, high-throughput NextGen (Illumina) sequencing has emerged as a powerful metagenomics tool for analyzing soil microbial community structure (Nkongolo and Narendrula-Kotha 2020). Therefore, the composition of the soil 16S microbial community, in the presence of mycorrhizal and non-mycorrhizal roots (using a mutant-based approach-see below) was investigated in a field-based P leaching study (Chapter 4).

A major challenge in AMF study is the establishment of non-mycorrhizal control treatments (Watts-Williams and Cavagnaro 2015). In most studies, non-mycorrhizal treatments have been established by sterilizing experimental soils and back-inoculating them with AMF inoculum (or not for non-mycorrhizal controls) and bacterial filtrates (Asghari and Cavagnaro 2012). The majority of previous experiments on the impact of AMF on soil P leaching have taken this approach (Bender and van der Heijden 2015; Asghari and Cavagnaro 2011; Corkidi et al. 2011; Köhl and van der Heijden 2016). Bacterial filtrates are used to equilibrate the soil microbial community to one that is similar to that of non-sterilized soils; however, the soil microbes still may require a long time to recover (Asghari and Cavagnaro 2011). The mycorrhiza-defective tomato mutant *rmc*, which reduces mycorrhizal colonization, was first described by Barker et al. (1998b) and has since been widely used in the study of AM in the glasshouse and field (Watts-Williams and Cavagnaro 2015). A key advantage of using these tomato genotypes is that soils do not need to be sterilized to eliminate AMF. This makes it possible to investigate the impact of AMF on P leaching in soils with the wider soil biota intact. This pair of tomato genotypes has been used under field conditions to study effects of AMF on soil ecology (Cavagnaro et al. 2006), plant growth and soil carbon dynamics (Bowles et al. 2016), and in greenhouse studies on soil N

leaching (Bowles et al. 2017a; Asghari and Cavagnaro 2012). However, to my knowledge, this approach has not been used to evaluate mycorrhizal effects on soil P leaching in the field. Therefore, these tomato pairs were used both under glasshouse and field conditions to study effects of AMF on soil P leaching in this study (Chapter 2, 3 and 4).

Soil P immobilized by AM involves P uptake by the external hyphal network of the fungi, and subsequent translocation and transfer to the host plants (Smith et al. 2008). The external hyphae of AMF absorb inorganic orthophosphate (Pi) from soil solution, and then the P is condensed into inorganic polyphosphate (PolyP) for long-distance translocation along the hyphae (Callow et al. 1978; Solaiman et al. 1999). The process of P acquisition by the hyphae of AMF is of central importance to AM reducing the available soil P pool that would otherwise be at risk of being lost. Therefore, understanding how soil P is taken up and stored in mycorrhizal external hyphae is an important question. Nuclear magnetic resonance (NMR) spectroscopy is a powerful tool for the study of the nature and molecular characteristics of chemically complex P species. In this project, ^{31}P NMR spectroscopy was used to investigate P metabolism in external mycelium of AMF (Chapter 5). While this work did not develop into a major theme in this project, some important results were found and so are presented herein.

1.2 Aims and objectives of the project

The project's main aim is to examine how AM impact on P cycling and leaching in soils by evaluating P dynamics in the plant, soil and leachate (Figure 1).

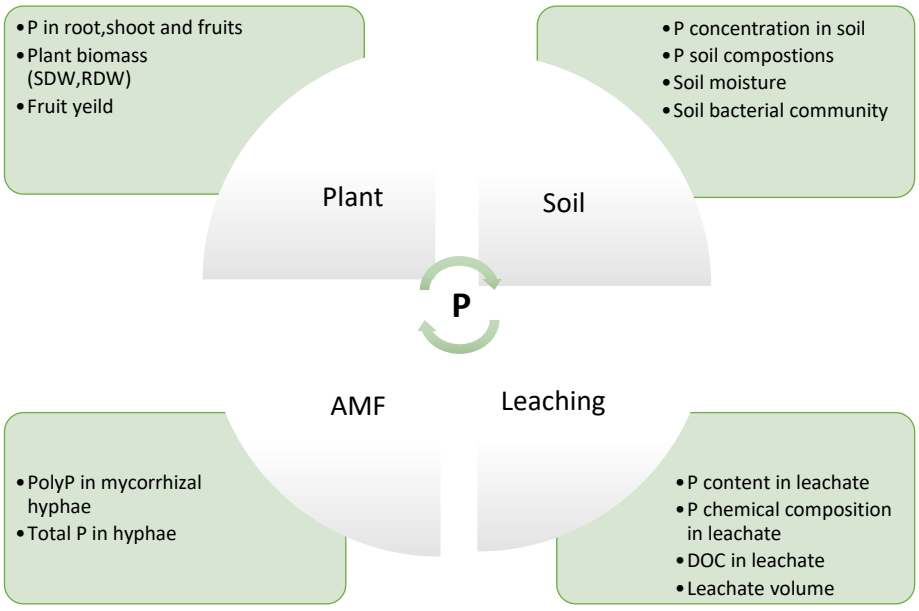

Figure 1. Conceptual diagram of soil P pool to investigate mycorrhizal effects on soil P leaching in this project.

The mycorrhizal-defective tomato mutant, *rmc* and its wild-type progenitor (76R) were used to establish mycorrhizal treatments in all leaching experiments. This approach enabled experiments to be undertaken using non-sterilized soils, both in the glasshouse and field.

The specific objectives of the project were to:

i. Determine the effects of arbuscular mycorrhizas on plant P uptake, plant growth and plant yield;
ii. Determine the effects of arbuscular mycorrhizas on the amount of P in leachate, chemical composition of the P in leachate, leachate volume;
iii. Determine the effects of arbuscular mycorrhizas on the amount of P soil, chemical composition of P soil after a leaching event;
iv. Evaluate the effects of arbuscular mycorrhizas on soil moisture, the soil bacterial community and their interaction with soil P leaching;
v. Characterize polyphosphate storage in mycorrhizal external mycelium using ^{31}P NMR spectroscopy.

1.3 Overall structure of the thesis

The body of this thesis consists of four experimental data chapters written in manuscript style, including two glasshouse-based microcosm experiments, a field-based experiment to investigate AMF effects on soil P cycling and leaching, and a NMR study to examine the polyphosphate storage in mycorrhizal hyphae (See Figure 2).

Chapter 2: Effects of plant roots and arbuscular mycorrhizas on soil phosphorus leaching.

This study focused on mycorrhizal effects on plant P, plant biomass, P composition in leachate and soil P under two different P levels.

-AM had a positive impacts on plant P and plant biomass;

-AM and roots increased reactive P and DOC leached;

-The total P and DOC leached strongly correlated.

Chapter 3: Root and mycorrhizal effects on soil nutrient loss are modulated by soil texture.

This chapter studied mycorrhizal and root effects on the amount and composition of soil P at different depths, P and DOC leached; and soil moisture for two different soil textures.

-Soil texture affected mycorrhizal colonization, P plant uptake, P and DOC leached;

-AM reduced P leached in sandy subtrate, associated with high DOC leached.

Chapter 4: Arbuscular mycorrhizal and field-grown tomatoes: a study of growth, phosphorus leaching, soil moisture and the wider soil microbial community.

A field-based experiment was conducted to investigate mycorrhizal effects on plant nutrients, plant biomass, P leached, soil moisture and soil bacterial community.

-AM increased fruit yield and nutrient concentrations;

-AM had no significant impact on P soil, soil moisture or soil16S bacterial community and P leached.

Chapter 5: A study on phosphorus uptake and storage in external mycelium of arbuscular mycorrhizal fungi.

A NMR study was conducted to examine polyphosphate storage in the external mycorrhizal mycelium.

-*Medicago truncatula* inoculated with *Rhizophagus irregularis* in the hyphae-root compartmented system created sufficient quantities of external hyphae for NMR analysis;

-Polyphosphate peaks were identified in the ^{31}P NMR spectra.

Figure 2. Thesis body structure

References

Adesemoye AO, Kloepper JW (2009). Plant-microbes interactions in enhanced fertilizer-use efficiency. Applied Microbiology and Biotechnology 85 (1):1-12. doi:10.1007/s00253-009-2196-0

Allen MF (2011). Linking water and nutrients through the vadose zone: A fungal interface between the soil and plant systems. Journal of arid land 3 (3):155-163. doi:10.3724/SP.J.1227.2011.00155

Artursson V, Finlay RD, Jansson JK (2006). Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environmental microbiology 8 (1):1-10. doi:10.1111/j.1462-2920.2005.00942.

Asghari HR, Cavagnaro TR (2011). Arbuscular mycorrhizas enhance plant interception of leached nutrients. Functional Plant Biology 38 (3):219-226. doi:10.1071/FP10180

Asghari HR, Cavagnaro TR (2012). Arbuscular mycorrhizas reduce nitrogen loss via leaching Ploss one 7 (1)

Asghari HR, Chittleborough DJ, Smith FA, Smith SE (2005). Influence of arbuscular mycorrhizal (AM) symbiosis on phosphorus leaching through soil cores. Plant and Soil 275 (1):181-193. doi:10.1007/s11104-005-1328-2

Atul-Nayyar A, Hamel C, Hanson K, Germida J (2009). The arbuscular mycorrhizal symbiosis links N mineralization to plant demand. Mycorrhiza 19 (4):239-246. doi:10.1007/s00572-008-0215-0

Augé RM (2004). Arbuscular mycorrhizae and soil/plant water relations. Canadian Journal of Soil Science 84 (4):373-381. doi:10.4141/S04-002

Baker JL, Campbell KL, Johnson HP, Hanway JJ (1975). Nitrate, phosphorus and sulfate in subsurface drainage water. Journal of Environmental Quality 4:406-412

Barea J-M, Azcón R, Azcón-Aguilar C (2002). Mycorrhizosphere interactions to improve plant fitness and soil quality. Antonie van Leeuwenhoek 81 (1):343-351. doi:10.1023/A:1020588701325

Barker SJ, Stummer B, Gao L, Dispain I, O'Connor PJ, Smith SE (1998). A mutant in Lycopersicon esculentum Mill. with highly reduced VA mycorrhizal colonization: isolation and preliminary characterisation. The Plant journal : for cell and molecular biology 15 (6):791-797. doi:10.1046/j.1365-313X.1998.00252.x

Bender SF, Conen F, Van der Heijden MGA (2014). Mycorrhizal effects on nutrient cycling, nutrient leaching and N2O production in experimental grassland. Soil Biology and Biochemistry 80 (Supplement C):283-292. doi:https://doi.org/10.1016/j.soilbio.2014.10.016

Bender SF, van der Heijden MGA (2015). Soil biota enhance agricultural sustainability by improving crop yield, nutrient uptake and reducing nitrogen leaching losses. Journal of Applied Ecology 52 (1):228-239. doi:10.1111/1365-2664.12351

Bolan NS (1991). A critial review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant and Soil 134:189-207

Bolan NS, Robson AD, Barrow NJ (1987). Effect of vesicular-arbuscular mycorrhiza on the availibility of iron phosphates to plants. Plant and Soil 99:401-410

Bowles TM, Barrios-Masias FH, Carlisle EA, Cavagnaro TR, Jackson LE (2016). Effects of arbuscular mycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. The Science of the total environment 566-567:1223-1234. doi:10.1016/j.scitotenv.2016.05.178

Bowles TM, Jackson LE, Cavagnaro TR (2017). Mycorrhizal fungi enhance plant nutrient acquisition and module nitrogen loss with variable water regimes. Global Change Biology. doi:10.1111/gcb.13884

Brailsford FL, Glanville HC, Golyshin PN, Johnes PJ, Yates CA, Jones DL (2019). Microbial uptake kinetics of dissolved organic carbon (DOC) compound groups from river water and sediments. Scientific reports 9 (1):11229-11211. doi:10.1038/s41598-019-47749-6

Brookes PC, Heckrath G, Smet JD, Hofman G, Vanderdeelen J (1997). Losses of phosphorus indrainage water. In:Tunney H, Carton OT, Brookes PC, Johnston AE (eds) Phosphorus loss from soil to Water. CAB International, Wallingford, pp 253-271

Callow JA, Capaccio LCM, Parish G, Tinker PB (1978). Detection and estimation of poly phosphate in vesicular and arbuscular mycorrhizas. New Phytologist 80 (1):125-134. doi:10.1111/j.1469-8137.1978.tb02272.x

Carpio LA, Davies FT, Jr., Arnold MA (2005). Arbuscular mycorrhizal fungi, organic and inorganic controlled-release fertilizers: effect on growth and leachate of container-grown bush morning glory (Ipomoea carnea ssp. fistulosa) under high production temperatures. Journal of the American Society for Horticultural Science 130 (1):131-139. doi:10.21273/JASHS.130.1.131

Carstensen J, Andersen JH, Gustafsson BG, Conley DJ (2014). Deoxygenation of the baltic sea during the last century. Proceedings of the National Academy of Sciences of the United States of America 111 (15):5628-5633. doi:10.1073/pnas.1323156111

Cavagnaro TR (2008). The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: A review. Plant and Soil 304 (1-2):315-325. doi:10.1007/s11104-008-9559-7

Cavagnaro TR, Bender SF, Asghari HR, van der Heijden MGA (2015). The role of arbuscular mycorrhizas in reducing soil nutrient loss. Trends in Plant Science 20 (5):283-290. doi:10.1016/j.tplants.2015.03.004

Cavagnaro TR, Jackson LE, Six J, Ferris H, Goyal S, Asami D, Scow KM (2006). Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. Plant and Soil 282 (1):209-225. doi:10.1007/s11104-005-5847-7

Cavagnaro TR, Smith FA, Smith SE, Jakobsen I (2005). Functional diversity in arbuscular mycorrhizas: Exploitation of soil patches with different phosphate enrichment differs among fungal species. Plant, Cell and Environment 28 (5):642-650. doi:10.1111/j.1365-3040.2005.01310.x

Chen GC, He ZL, Stoffella PJ, Yang XE, Yu S, Calvert D (2006). Use of dolomite phosphate rock (DPR) fertilizers to reduce phosphorus leaching from sandy soil. Environmental pollution (1987) 139 (1):176-182. doi:10.1016/j.envpol.2004.12.016

Clark RB, Zeto SK (2008). Mineral acquisition by arbuscular mycorrhizas plants. Journal of Plant Nutrition 23 (7):867-902. doi: 10.1080/01904160009382068

Cordell D, Drangert JO, White S (2009). The story of phosphorus: Global food security and food for thought. Global Environmental Change 19 (2):292-305. doi:10.1016/j.gloenvcha.2008.10.009

Corkidi L, Merhaut DJ, Allen EB, Downer J, Bohn J, Evans M (2011). Effects of mycorrhizal colonization on nitrogen and phosphorus leaching from nursery containers. HortScience 46 (11):1472-1479

Dai Z, Liu G, Chen H, Chen C, Wang J, Ai S, Wei D, Li D, Ma B, Tang C, Brookes PC, Xu J (2020). Long-term nutrient inputs shift soil microbial functional profiles of phosphorus cycling in diverse agroecosystems. The ISME Journal 14 (3):757-770. doi:10.1038/s41396-019-0567-9

David DD, Philip EP, Yair S-H (2000). Application of in vitro methods to study carbon uptake and transport by AM fungi. Plant and soil 226 (2):255-261. doi:10.1023/A:1026466802354

Dong L, Jonathan OS, Pascal ES, Shahjahan A, Mazahirali A, Mohammed SA, Stephanie K, Christiane H-J, Jörg ED (2012). Dissolved organic carbon influences microbial community composition and diversity in managed aquifer recharge systems. Applied and Environmental Microbiology 78 (19):6819-6828. doi:10.1128/AEM.01223-12

Duffková R, Fučík P, Jurkovská L, Janoušková M (2019). Experimental evaluation of the potential of arbuscular mycorrhiza to modify nutrient leaching in three arable soils located on one slope. Applied soil ecology : a section of Agriculture, ecosystems & environment 143:116-125. doi:10.1016/j.apsoil.2019.06.001

El Maaloum S, Elabed A, Alaoui-Talibi ZE, Meddich A, Filali-Maltouf A, Douira A, Ibnsouda-Koraichi S, Amir S, El Modafar C (2020). Effect of arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria consortia associated with phospho-compost on phosphorus solubilization and growth of tomato seedlings (*Solanum lycopersicum* L.). Communications in soil science and plant analysis 51 (5):622-634. doi:10.1080/00103624.2020.1729376

Frossard E, Condron LM, Oberson A, Sinaj S, Fardeau JC (2000). Processes governing phosphorus availability in temperate soils. Journal of Environmental Quality 29 (1):15-23. doi:10.2134/jeq2000.00472425002900010003x

Gosling P, Mead A, Proctor M, Hammond JP, Bending GD (2013). Contrasting arbuscular mycorrhizal communities colonizing different host plants show a similar response to a soil phosphorus concentration gradient. The New phytologist 198 (2):546-556. doi:10.1111/nph.12169

He Y, Yang R, Lei G, Li M, Li T, Zhan F, Li Y (2021). Arbuscular mycorrhizal fungus induced decrease in phosphorus loss due to leaching in red soils under simulated heavy rainfall. Journal of soils and sediments 21 (2):881. doi:10.1007/s11368-020-02849-z

Herzog F, Prasuhn V, Spiess E, Richner W (2008). Environmental cross-compliance mitigates nitrogen and phosphorus pollution from Swiss agriculture. Environmental Science and Policy 11 (7):655-668. doi:10.1016/j.envsci.2008.06.003

Hetrick BAD (1989). Acquisition of phosphorus by VA mycorrhizal fungi and the growth responses of their host plants. In: Boddy L, Marchant R, Read DJ (eds) Nitrogen, phosphorus and sulphur utilization by fungi. Cambridge University Press, Cambridge, pp 205-226

Huang PM, Li Y, Sumner ME (2011). Handbook of soil sciences resource management and environmental impacts. Handbook of Soil Science, 2nd ed. edn. CRC Press, Boca Raton, Fla

Jakobsen I, Abbott LK, Robson AD (1992). External hyphae of vesicular-arbuscular mycorrhizal fungi associated with Trifolium subterraneum L. . Spread of hyphae and phosphorus Inflow into roots. The New Phytologist 120 (3):371-379. doi:10.1111/j.1469-8137.1992.tb01077.x

Javot H, Penmetsa RV, Terzaghi N, Cook DR, Harrison MJ (2007). A Medicago truncatula phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. Proceedings of the National Academy of Sciences 104 (5):1720

Jennifer LP, David B, Roger TK, Andrew GS (2002). Mycorrhizal infection and high soil phosphorus improve vegetative growth and the female and male functions in tomato. The New phytologist 154 (1):255-264. doi:10.1046/j.1469-8137.2002.00366.x

Jiang XJ, Liu W, Chen C, Liu J, Yuan Z-Q, Jin B, Yu X (2018). Effects of three morphometric features of roots on soil water flow behavior in three sites in China. Geoderma 320:161-171. doi:10.1016/j.geoderma.2018.01.035

Kalbitz K, Solinger S, Park JH, Michalzik B, Matzner E (2000). Controls on the dynamics of dissolved organic matter in soils : A review. Soil science 165 (4):277-304. doi:10.1097/00010694-200004000-00001

Kang J, Amoozegar A, Hesterberg D, Osmond DL (2011). Phosphorus leaching in a sandy soil as affected by organic and inorganic fertilizer sources. Geoderma 161 (3):194-201. doi:10.1016/j.geoderma.2010.12.019

Kim KY, Jordan D, McDonald GA (1997). Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biology and fertility of soils 26 (2):79-87. doi:10.1007/s003740050347

Köhl L, van der Heijden MGA (2016). Arbuscular mycorrhizal fungal species differ in their effect on nutrient leaching. Soil Biology and Biochemistry 94:191-199. doi:10.1016/j.soilbio.2015.11.019

Kohl L, van der Heijden MGA, Oehl F (2014). Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota.(Report). Ecological Applications 24 (7)

Laird D, Fleming P, Wang B, Horton R, Karlen D (2010). Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma 158 (3):436-442. doi:https://doi.org/10.1016/j.geoderma.2010.05.012

Lehmann A, Veresoglou SD, Leifheit EF, Rillig MC (2014). Arbuscular mycorrhizal influence on zinc nutrition in crop plants - A meta-analysis. Soil Biology and Biochemistry 69:123-131. doi:10.1016/j.soilbio.2013.11.001

Lehmann J, Schroth G (2003). Nutrient leaching. In: Schroth G, Sinclair FL (eds) Tree, crops and soil fertility. CAB International, Wallingford, UK, pp 151–166.

Leung AK, Garg A, Coo JL, Ng CWW, Hau BCH (2015). Effects of the roots of Cynodon dactylon and Schefflera heptaphylla on water infiltration rate and soil hydraulic conductivity:effects of plant roots on infiltration characteristics and suction. Hydrological Processes 29 (15):3342-3354. doi:10.1002/hyp.10452

Li XL, George E, Marschner H (1991). Extension of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil. Plant and Soil 136 (1):41-48. doi:10.1007/BF02465218

Lindegren M, Persson P (2010). Competitive adsorption involving phosphate and benzenecarboxylic acids on goethite—Effects of molecular structures. Journal of colloid and interface science 343 (1):263-270. doi:10.1016/j.jcis.2009.11.040

Liu X-P, Bi Q-F, Qiu L-L, Li K-J, Yang X-R, Lin X-Y (2019). Increased risk of phosphorus and metal leaching from paddy soils after excessive manure application: Insights from a mesocosm study. The Science of the total environment 666:778-785. doi:10.1016/j.scitotenv.2019.02.072

Marc IS (2015). The composition, leaching, and sorption behavior of some alternative sources of phosphorus for soils. Ambio 44 (S2):S207-S216. doi:10.1007/s13280-014-0615-7

Marschner H, Dell B (1994). Nutrient uptake in mycorrhizal symbiosis. Plant and Soil 159 (1):89-102. doi:10.1007/BF00000098

Marschner P, Crowley D, Lieberei R (2001). Arbuscular mycorrhizal infection changes the bacterial 16 S rDNA community composition in the rhizosphere of maize. Mycorrhiza 11 (6):297-302. doi:10.1007/s00572-001-0136-7

Martinez-Garcia LB, De Deyn GB, Pugnaire FI, Kothamasi D, van der Heijden MGA (2017) Symbiotic soil fungi enhance ecosystem resilience to climate change. Global change biology 23 (12):5228-5236. doi:10.1111/gcb.13785

Mathur N, Vyas A (1999). Improved biomass production, nutrient uptake and establishment of in vitro raised Ziziphus mauritiana by VA mycorrhiza. Journal of plant physiology 155 (1):129-132. doi:10.1016/S0176-1617(99)80153-9

McDowell RW, Worth W, Carrick S (2021). Evidence for the leaching of dissolved organic phosphorus to depth. The Science of the total environment 755 (Pt 1):142392-142392. doi:10.1016/j.scitotenv.2020.142392

McLaughlin M J FIRaTAR (1991). Operation of the phosphorus , sulphur and nitrogen cycles. In: Barson RMGaMM (ed) Australia's renewable resources : sustainability and global change Bureau of rural resources, Canbera, Autralia, pp 67-116

Nacoon S, Jogloy S, Riddech N, Mongkolthanaruk W, Kuyper TW, Boonlue S (2020) Interaction between phosphate solubilizing bacteria and arbuscular mycorrhizal fungi on growth

promotion and tuber inulin content of Helianthus tuberosus L. Scientific reports 10 (1):4916-4916. doi:10.1038/s41598-020-61846-x

Nelson NO, Parsons JE, Mikkelsen RL (2005). Field-scale evaluation of phosphorus leaching in acid sandy soils receiving swine waste. Journal of environmental quality 34 (6):2024-2035. doi:10.2134/jeq2004.0445

Neumann A, Torstensson G, Aronsson H (2012). Nitrogen and phosphorus leaching losses from potatoes with diiferent harvest times and following crops. Field crops research 133:130-138

Nkongolo KK, Narendrula-Kotha R (2020). Advances in monitoring soil microbial community dynamic and function. Journal of applied genetics 61 (2):249-263. doi:10.1007/s13353-020-00549-5

Nuccio EE, Hodge A, Pett-Ridge J, Herman DJ, Weber PK, Firestone MK (2013). An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. Environmental microbiology 15 (6):1870-1881. doi:10.1111/1462-2920.12081

Nye PH, Tinker PB (1977). Solute movement in soil-root systems. University of California Press.

Parihar M, Meena V, Mishra P, Rakshit A, Choudhary M, Yadav R, Rana K, Bisht J (2019) Arbuscular mycorrhiza: a viable strategy for soil nutrient loss reduction. Archives of Microbiology 201 (6):723-735. doi:10.1007/s00203-019-01653-9

Pote D, Daniel T, Delaune P (2009). Total phosphorus and dissolved phosphorus in water samples. In: Kovar J, Pierzynski J (eds) Methods of phosphorus analysis for soils, sediment,residuals and waters. Virginia tech University, Blacksburg, VA, USA, pp 113-117

Raghothama K, Karthikeyan A (2005). Phosphate acquisition. An International Journal on Plant-Soil Relationships 274 (1):37-49. doi:10.1007/s11104-004-2005-6

Réka N, David D, Nikolaus A, Iver J, Marcel B (2009). Mycorrhizal phosphate uptake pathway in tomato is phosphorus-repressible and transcriptionally regulated. The New phytologist 181 (4):950-959. doi:10.1111/j.1469-8137.2008.02721.x

Rillig MC (2004). Arbuscular mycorrhizae and terrestrial ecosystem processes. vol 7. Oxford, UK. doi:10.1111/j.1461-0248.2004.00620.x

Rillig MC, Hoye AT, Carran A (2006a). Minimal direct contribution of arbuscular mycorrhizal fungi to DOC leaching in grassland through losses of glomalin-related soil protein. Soil Biology and Biochemistry 38 (9):2967-2970. doi:10.1016/j.soilbio.2006.03.022

Rillig MC, Mummey DL (2006). Mycorrhizas and soil structure. New Phytologist 171 (1):41-53. doi:10.1111/j.1469-8137.2006.01750.x

Rillig MC, Mummey DL, Ramsey PW, Klironomos JN, Gannon JE (2006b). Phylogeny of arbuscular mycorrhizal fungi predicts community composition of symbiosis-associated bacteria. FEMS microbiology ecology 57 (3):389-395. doi:10.1111/j.1574-6941.2006.00129.x

Roberts TL, Johnston AE (2015). Phosphorus use efficiency and management in agriculture. Resources, conservation and recycling 105:275-281. doi:10.1016/j.resconrec.2015.09.013

Roberts WM, George TS, Stutter MI, Louro A, Ali M, Haygarth PM (2020). Phosphorus leaching from riparian soils with differing management histories under three grass species. Journal of environmental quality 49 (1):74-84. doi:10.1002/jeq2.20037

Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M, Pascale SD, Bonini P, Colla G (2015). Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Scientia horticulturae 196:91-108. doi:10.1016/j.scienta.2015.09.002

Scott JT, Lambie SM, Stevenson BA, Schipper LA, Parfitt RL, McGill AC (2015). Carbon and nitrogen leaching under high and low phosphate fertility pasture with increasing nitrogen inputs. Agriculture, ecosystems & environment 202:139-147. doi:10.1016/j.agee.2014.12.021

Sharpley AN, Rekolainen S (1997). Phosphorus in agriculture and its enviromental implications In: Tunney H, Carton OT, Brookes PC, Johnston AE (eds) Phosphorus loss from soil to water. CAB International, Wallingford, pp 1-53

Sims JT, Simard RR, Joern BC (1998). Phosphorus loss in agricultural drainage: historical perspective and current research. Journal of Environmental Quality 27 (2):277-293

Smith SE, Iver J, Mette G, Andrew S (2011). Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant physiology (Bethesda) 156 (3):1050-1057. doi:10.1104/pp.111.174581

Smith SE, Read D (2008). Mycorrhizal symbiosis, third ed. Academic Press, Elsevier Ltd., Oxford

Smith SE, Smith FA (2011). Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annual Review of Plant Biology, vol 62. doi:10.1146/annurev-arplant-042110-103846

Solaiman MZ, Ezawa T, Kojima T, Saito M (1999). Polyphosphates in intraradical and extraradical hyphae of an arbuscular mycorrhizal gungus, Gigaspora margarita. Applied and Environmental Microbiology 65 (12):5604

Soudzilovskaia NA, van der Heijden MGA, Cornelissen JHC, Makarov MI, Onipchenko VG, Maslov MN, Akhmetzhanova AA (2015). Quantitative assessment of the differential impacts of arbuscular and ectomycorrhiza on soil carbon cycling. The New phytologist 208 (1):280-293

Stutter MI (2015). The composition, leaching, and sorption behavior of some alternative sources of phosphorus for soils. Ambio 44 (S2):S207-S216. doi:10.1007/s13280-014-0615-7

Tinker PB (1978). Effects of vesicula-arbuscular mycorrhizas on plant nutrition and plant growth Physiologie Vegetable 16:743-751

Treseder KK (2013). The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. Plant and soil 371 (1/2):1-13. doi:10.1007/s11104-013-1681-5

Ulen B (1999). Leaching and balances of phosphorus and other nutrients in lysimeters after application of organic manures or fertilizers. Soil use and management 15:56-61

van der Heijden MGA (2010). Mycorrhizal fungi reduce nutrient loss from model grassland ecosystems. Ecology 91 (4):1163-1171. doi:10.1890/09-0336.1

van der Heijden MGA, Wiemken A, Sanders IR (2003). Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plant. New Phytologist 157 (3):569-578. doi:10.1046/j.1469-8137.2003.00688.x

Vance CP (2001). Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. Plant Physiology 127 (2):390-397. doi:10.1104/pp.127.2.390

Verbruggen E, Kiers ET, Bakelaar PNC, Röling WFM, van der Heijden MGA (2012). Provision of contrasting ecosystem services by soil communities from different agricultural fields. Plant and Soil 350 (1-2):43-55. doi:10.1007/s11104-011-0828-5

Wardle DA, Walker LR, Bardgett RD (2004). Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305 (5683):509-513. doi:10.1126/science.1098778

Watts-Williams SJ, Cavagnaro TR (2014). Nutrient interactions and arbuscular mycorrhizas: a meta-analysis of a mycorrhiza-defective mutant and wild-type tomato genotype pair. Plant and Soil 384 (1-2):79-92. doi:10.1007/s11104-014-2140-7

Watts-Williams SJ, Cavagnaro TR (2015). Using mycorrhiza-defective mutant genotypes of non-legume plant species to study the formation and functioning of arbuscular mycorrhiza: a review. Mycorrhiza 25 (8):587-597. doi:10.1007/s00572-015-0639-2

Watts-Williams SJ, Tyerman SD, Cavagnaro TR (2017). The dual benefit of arbuscular mycorrhizal fungi under soil zinc deficiency and toxicity: linking plant physiology and gene expression. Plant and Soil 420 (1-2):375-388. doi:10.1007/s11104-017-3409-4

Zhang H, Liu T, Wang Y, Tang M (2019). Exogenous arbuscular mycorrhizal fungi increase soil organic carbon and change microbial community in poplar rhizosphere. Plant, soil and environment 65 (No. 3):152-158. doi:10.17221/2/2019-PSE

Zhang S, Yu X, Rillig M (2020). Arbuscular mycorrhiza contributes to the control of phosphorus loss in paddy fields. Plant and Soil 447 (1-2):623-636. doi:10.1007/s11104-019-04394-2

Ziane H, Meddad-Hamza A, Beddiar A, Gianinazzi S (2017). Effects of arbuscular mycorrhizal fungi and fertilization levels on industrial tomato growth and production. International journal of agriculture and biology 19 (2):341-347. doi:10.17957/IJAB/15.0287

Zouari I, Salvioli A, Chialva M, Novero M, Miozzi L, Tenore G, Bagnaresi P, Bonfante P (2014). From root to fruit: RNA-Seq analysis shows that arbuscular mycorrhizal symbiosis may affect tomato fruit metabolism. BMC genomics 15 (1):221-221. doi:10.1186/1471-2164-15-221

Chapter 2-Effects of plant roots and arbuscular mycorrhizas on soil phosphorus leaching

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Effects of plant roots and arbuscular mycorrhizas on soil phosphorus leaching

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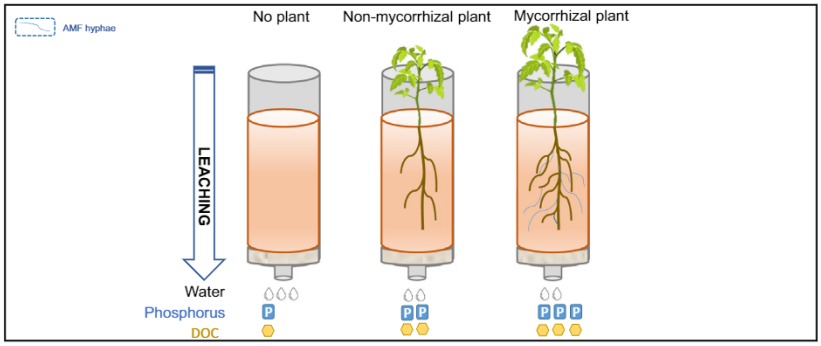
HIGHLIGHTS

- Non-sterile soils with unplanted, or mycorrhizal or non-mycorrhizal plants were studied.
- Mycorrhizal plants corresponded to the most leached total and reactive P, and dissolved organic C.
- Leached total P and dissolved organic carbon were strongly correlated.

GRAPHICAL ABSTRACT



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ABSTRACT

While the impact of arbuscular mycorrhizal fungi (AMF) on phosphorus (P) uptake is well understood, the mechanism(s) of how these fungi affect P leaching from soil is still unclear. Here we present results of a study in which we grew a mycorrhiza-defective tomato (*Solanum lycopersicum* L.) genotype (named *rmc*) and its mycorrhizal wild-type progenitor (named 76R) in microcosms containing non-sterile soil, to examine the influence of roots and AMF on P leaching. More P was leached from the planted microcosms as compared to the plant-free controls. Further, although there was more plant biomass and greater P uptake in the mycorrhizal plant treatments, these treatments were associated with the most leaching of total P, reactive P, and dissolved organic carbon (DOC). There was a strong correlation between the total P and DOC leached, suggesting that root and fungal exudates may have affected P leaching. These findings provide new insights into the impact of roots and AMF on nutrient leaching in soils.

1. Introduction

The availability of phosphorus (P) in many soils is low due to the propensity for P to bind to soil particles and form precipitates with soil cations (White, 2009). As a result, it is estimated that only 10–30% of P applied as fertilizer is taken up by plants (McLaughlin, 1991).

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Consequently, agricultural application rates of P can be high (Frossard et al., 2000; Ozanne et al., 1961). Although P leaching from soils has long been considered negligible in most situations (Brookes et al., 1997; Bender and van der Heijden, 2015), it can occur where rates of P application are high and plant assimilation is low. Since the movement of P from soil into waterways can lead to eutrophication and biodiversity loss (Sharpley and Rekolainen, 1997), research into ways to mitigate soil P loss has been longstanding (Reid et al., 2018).

Arbuscular mycorrhizal fungi (AMF) are a widespread group of soil fungi that establish a symbiotic association with the roots of 80% of terrestrial plant species (Smith and Smith, 2011). AMF can increase plant P acquisition (Marschner and Dell, 1994; Cavagnaro, 2008), which may result in a reduced available soil P pool that would otherwise be at risk of being lost; thus, AMF may reduce soil P leaching (Asghari et al., 2005; Bender et al., 2015). The reduction in P lost through leaching associated with AMF is explained mainly by the extension of the fungal hyphae, efficient P plant uptake, and P immobilization in plant and fungi biomass (Asghari and Cavagnaro, 2011; Cavagnaro et al., 2015). However, AMF do not always decrease P leaching, with some previous studies reporting no effects on P leaching (Verbruggen et al., 2012; Köhl and van der Heijden, 2016). The effects of AMF on leaching may depend on various AMF-mediated changes in the chemical composition of soil leachates, but this topic is not well-studied. This is an important gap in knowledge, given that the chemical form of P can impact on its behavior in the environment (Turner et al., 2002). Many types of P compounds can be leached from soil including "reactive" P forms, which are directly available to plants (primarily dissolved phosphate) and "unreactive" P forms (soluble and particulate organic P compounds, polyphosphates and particulate inorganic materials) (Pote et al., 2009). Leaching of unreactive P can represent 60–90% of total P leached (Bender et al., 2015; Ulen, 1999; Neumann et al., 2012). In addition, previous studies showed that AMF can reduce unreactive P in leachates (Bender and van der Heijden, 2015). Because of the potential contribution of various types of P compounds in the leachate and the potential effect of AMF on reducing these P compounds, it is necessary to identify the composition of all P leaching under AMF-plant systems to ascertain whether AMF can reduce overall P soil loss or only some P forms.

The impact of AMF on soil nutrient loss via leaching has been mostly investigated in sterilized soil systems which make use of bacterial filtrates to establish the non-mycorrhizal treatments (Köhl and van der Heijden, 2016; Bender et al., 2015; Asghari and Cavagnaro, 2011; Corkidi et al., 2011; Asghari et al., 2005). The aim of bacterial filtrates is to reproduce soil microbial communities similar to that of non-sterilized soils. However, the soil microbes may require a long time to recover (Asghari and Cavagnaro, 2011). Mycorrhiza-defective tomato mutant genotypes have been developed for research purposes to investigate many aspects of soil-plant processes mediated by rhizosphere microbial communities (Watts-Williams and Cavagnaro, 2014). A key advantage of these genotypes as tools is that they are relatively non-invasive since AMF do not have to be eliminated from the system (Rillig et al., 2008). So far, this approach has been used to evaluate the effect of AMF on N leaching (Asghari and Cavagnaro, 2012; Bowles et al., 2017) but not yet on P leaching.

The influence of AMF on P leaching in sterilized soil has been well studied; the same is not true of unsterilized soil. Moreover, most studies of AMF impacts on soil nutrient loss via leaching have not considered the nature of the P leached (i.e. total P versus reactive P). Therefore, the goal of this study was to test the hypotheses that AMF can reduce the P leached in the non-sterile soil by using mycorrhiza-defective tomato mutant genotypes. Specifically, we hypothesized that:

i) Colonization of roots by AMF would increase the mobility of P in the soil, and thus increase plant P uptake and growth compared to a non-colonized plant, and
ii) Colonization of roots by AMF would reduce the leachate volume, the amount of the P leached, and modify the composition of P leached, compared to a pot containing a non-colonized plant.

2. Materials and methods

Leaching experiments were conducted in a glasshouse at the University of Adelaide's Waite Campus (Adelaide, South Australia, Australia) from July to August 2018. A mycorrhiza-defective tomato (*Solanum lycopersicum* L.) mutant with reduced mycorrhizal colonization (named *rmc* hereafter), and its mycorrhizal wild type progenitor (named 76R hereafter) (Barker et al., 1998) were used to establish mycorrhizal and non-mycorrhizal control treatments in the non-sterile soil. The experiment was set up with three P levels (see below), the two tomato genotypes and a plant-free control treatment; there were five replicates per treatment and a total of 45 microcosms.

2.1. Microcosms, soil and nutrient addition

The microcosms used in this experiment were constructed with PVC pipe (90 mm diameter × 350 mm height), 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 easy collection of leachates (Bowles et al., 2017). 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. All microcosms were then filled with 2 kg of a soil/sand mixture including 100 g of AMF inoculum per pot, and amended with P, as follows.

The soil used was collected from the 0–10 cm layer of the University of Adelaide's Waite Campus Arboretum, South Australia. The soil at this site is a fine sandy loam, Urrbrae red-brown earth (Alfisol) with total P concentration of 248 ± 4 mg kg^{-1} dry soil and plant-available (Colwell) P concentration of 16 ± 0.5 mg kg^{-1} dry soil (see below for analytical methods). The soil was air-dried and sieved to <2 mm, and then mixed with fine sand in a ratio of 70:30 (soil/sand *w*/*w*). This soil/sand mixture provides a substrate conducive to uniform leaching, and facilitates ready extraction of roots at the time of harvest (Bowles et al., 2017); this mixture is referred to as 'soil' hereafter. The field capacity of the soil was determined using a sintered glass funnel connected to a 1 m water column ($\Psi_m = -10$ kPa) (Cavagnaro, 2016). 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 h and weighed. The soil was then dried at 105 °C for 48 h and gravimetric moisture content calculated, following Ngo and Cavagnaro (2018). The gravimetric moisture content at field capacity was 0.22 g water g^{-1} dry soil. The total and plant-available (Colwell) P concentrations of the soil were 202 ± 4 mg kg^{-1} dry soil and 12 ± 0.5 mg kg^{-1} dry soil, respectively.

An AMF inoculum was derived from single-species pot cultures of *Rhizophagus irregularis* WFVAM10 (formerly named *Glomus intraradices*). The AMF inoculum was a pot culture containing a mixture of dry soil, spores, external hyphae and root fragments of *Trifolium subterraneum* L. (clover) cv. Mt. Barker.

Three P addition treatments were established by mixing $K_2HPO_4.3H_2O$ dissolved in 50 mL of reverse osmosis (RO) water, thoroughly through the soil. This method of P addition was found to provide a uniform mixture in a preliminary experiment (data not shown). The three rates of P application were 0, 10 and 20 mg P kg^{-1} soil and gave a final plant-available (Colwell) P concentration of: 12 ± 0.36, 20 ± 0.47 and 28 ± 0.5 mg P kg^{-1} dry soil analysed immediately after adding fertilizer.

Seeds of the 76R and *rmc* tomato genotypes were surface sterilized with a 10% sodium hypochlorite solution, rinsed with RO water, and then sown into coarse sand for germination. One week later the seedlings with fully expanded cotyledons were transplanted into the microcosms (one seedling per microcosm).

Following planting, the microcosms were watered with RO water to 75% of WHC (by weight) to provide sufficient moisture to support plant growth, but avoiding water being leached from the microcosms during the plant growth phase of the experiment. Plants were grown in a glasshouse with 14.5/9.5 h light/dark cycle; mean minimum and maximum

temperatures were 16.8 and 22.5° C, respectively; mean minimum and maximum humidity were 31.5% and 85.8%, respectively. One week after planting all plants were supplied with 30 mL of a modified Long-Ashton nutrient solution without P (Cavagnaro et al., 2001), then 10 mL of the same solution weekly, thereafter. Three weeks after transplanting, 7 mg P as $K_2HPO_4.3H_2O$, and 20 mg N as NH_4NO_3 were added (in the irrigation water) to all pots following the appearance of foliar symptoms of P and N deficiency.

2.2. Harvesting and leachate collection

Six weeks after planting into the microcosms, the shoots of plants were cut at the soil surface (to eliminate water loss via transpiration), and immediately after, the columns were flushed slowly with a total of 700 mL of RO water, equivalent to a rainfall event of 110 mm (Asghari and Cavagnaro, 2012), in order to leach soil nutrients from the column. Aliquots of 200 mL of water at a time were added to the surface of the soil. This represents an equivalent addition of 31.4 mm rainfall, but due to rapid initial infiltration, the depth of free water on the surface was always less than this. Subsequent aliquots were added when there was no free water remaining on the surface and the full 700 mL of water was added within a period of approximately 12 h. The leachates were collected from the microcosms over a period of 48 h by which time outflow had ceased.

2.3. Leachate analysis

The volume of leachate collected was recorded, and leachate pH, total P, molybdate-blue reactive P and dissolved organic carbon (DOC), quantified, as follows. The pH was measured directly on the leachates using a hand-held electrical conductivity/pH meter (WP-81, TPS Pty Ltd.). Total P in leachates was measured directly on leachate using inductively coupled plasma-optical emission spectrometry ICP-OES (Avio 200, Perkin Elmer). Molybdate-blue reactive P was measured colorimetrically using a Multiskan Go (Thermo Scientific) plate reader (Murphy and Riley, 1962). The concentration of DOC in leachates was measured directly using a Shimadzu total organic carbon and total nitrogen analyser. These data, together with leachate volumes were used to calculate total amounts of P (total P and reactive P) and DOC leached. The values of unreactive P were calculated for each replicate by subtracting the reactive P from total P. These values were then used to calculate the means and standard errors, following the method of (Bender et al., 2015)

2.4. Plant biomass and soil analysis

Immediately following the leaching event, all soil was removed from the soil cores and a representative 100 g sample was taken for determination of plant-available (Colwell) P and total P as follows. The concentration of total P in soil samples was analysed using an Avio 200 ICP-OES (Perkin Elmer), following digestion with nitric acid and hydrochloric acid (Wheal et al., 2011). The concentration of plant-available (Colwell) P in soil samples was determined using a modification of Colwell (1963) (extraction with 0.5 $NaHCO_3$ at a soil:extractant ratio of 1:100 and 16 h shaking) followed by colorimetric analysis (Murphy and Riley, 1962).

The roots were carefully washed free of the remaining soil with RO water and fresh weights determined. A subsample (of known weight) of plant roots was cleared with 10% KOH (*w*/*v*) and stained with 5% ink in vinegar (Vierheilig et al., 1998). Mycorrhizal colonization of the roots was then determined using the gridline intersect method for at least 100 intersections (Giovannetti and Mosse, 1980). All remaining plant material was dried at 60 °C for 48 h, before shoot dry weights (SDW) and root dry weights (RDW) were determined. Dried shoots and roots were then ground to a fine powder. The concentration of P in shoots and roots was analysed using an Avio 200 ICP-OES (Perkin Elmer), following digestion with nitric acid and hydrogen peroxide (Wheal et al., 2011).

2.5. Statistical analysis

All data were analysed using GENSTAT (VSN International (2017) for *Windows* 18th Edition. VSN International, Hemel Hempstead, UK. Web page: Genstat.co.uk). Data were checked for the assumption of normality by the residual plots. Two-way analysis of variance (ANOVA) was performed with the factors P addition treatments and mycorrhizal plant/non-mycorrhizal plant (for the plant biomass parameters) or no plant/mycorrhizal plant/non-mycorrhizal plant (for soil and leachate parameters). Where there were significant interactions, means were compared using the Fisher's Protected LSD method ($P<0.05$). The correlation between total P content and DOC in the leachates was tested by regression analysis using GENSTAT (simple linear regression).

3. Results

3.1. Mycorrhizal colonization, plant growth and nutrient uptake

Whereas roots of the 76R plants in all treatments were colonized by AMF (37 ± 3% of root length colonized on average across all treatments) (Fig. 1a), those of the *rmc* genotype were not colonized. Colonization of the 76R plants was impacted by soil P addition with colonization higher where P was added to the soil.

Mycorrhizal colonization and soil P addition had a significant impact on plant biomass (Fig. 1b) and P uptake (contents) (Fig. 1c). Specifically, the root and shoot dry weights, and P content of the mycorrhizal plants were greater than that of the non-mycorrhizal plants, irrespective of soil P addition treatment (the only exception was the shoot P content in the P20 treatments c). Additionally, in response to soil P supply, plant biomass and P contents were higher in the P20 treatment (irrespective of mycorrhizal treatment) than in the P0 and P10 treatments. (See Table 1).

3.2. Leachate volume and nutrient content

After 48 h, all added water had infiltrated the soil surface in all treatments. The volume of leachate collected at the end of the experiment of the P10 treatment was higher than that of 0P and 20P treatment. There was no significant difference in leachate volume between mycorrhizal plants and non-mycorrhizal plants but leachate volume was higher in the absence of a plant (Fig. 2a). Leachate pH (Fig. 2b) ranged 7.0 to 7.4, and was higher when a mycorrhizal plant (and non-mycorrhizal plant at P20 only) was present compared to no plant present.

The impact of the plant and P addition treatments on P leaching in this experiment was complex. Data for total P, reactive P, and (by subtraction) unreactive P are presented in Fig. 3a. Total P was lowest and relatively unaffected by P addition treatment in the plant-free treatments, but it increased with increasing P addition in mycorrhizal plant microcosms. The total P leached from non-mycorrhizal plant microcosms was not different between P0 and P10 but increased at P20. This response was largely driven by reactive P, rather than un-reactive P in the leachates; reactive P followed the same pattern as total P in the leachates, whereas unreactive P was relatively constant across all treatments. As a result, there were contrasting ratios of reactive P to unreactive P in the leachates (Fig. 3b). This ratio was significant lower in the plant-free control pots than in plant pots ($P<0.001$); there was no significant difference in this ratio between mycorrhizal plants and non-mycorrhizal plants (Table 2). This ratio was higher for P20 treatments than for the P0 and P10 treatments.

Similarly to the total P in the leachates, the DOC content was lowest and relatively unaffected by P addition treatment in the plant-free treatments, but it increased with increasing P addition in mycorrhizal plant microcosms. The DOC content of the non-mycorrhizal plant microcosms was not different between P0 and P10 but increased at P20 (Fig. 4a). Interestingly, DOC content in leachate was correlated with the total P content in the leachate ($R^2 = 0.76$, $P<0.001$, $n = 45$). The correlation was

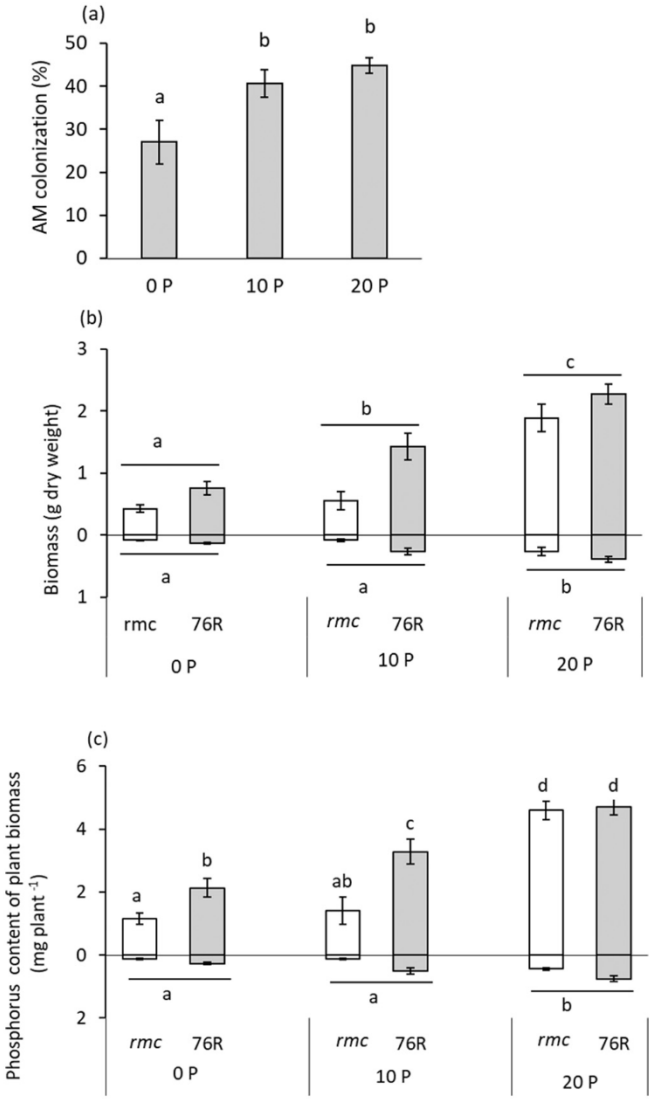

Fig. 1. Arbuscular mycorrhizal colonization of 76R roots (a), mean shoot (above x-axis) and root (below x-axis) dry weight (b), and plant phosphorus (P) content of the *rmc* (white bar) and the 76R (grey bar) genotypes of tomato (c), following the application of P treatments. 76R and *rmc* are mycorrhizal and mycorrhiza-defective tomato genotypes. 0P, 10P and 20 P are P treatments at 0, 10 and 20 mg P kg^{-1} dry soil. Values are mean ± SE, $n = 5$. Means followed by the same letter are not significantly different at the $P<0.05$ level.

stronger for the reactive P content in the leachate ($R^2 = 0.68$, $P<0.001$, $n = 45$) than the unreactive P content ($R^2 = 0.29$, $P<0.001$, $n = 45$) (Fig. 4b).

3.3. Total and plant-available (Colwell) soil P

In general, the amount of total P, including unreactive P and reactive P remaining in soil after leaching did not differ between mycorrhizal and non-mycorrhizal treatments (the only exception was the reactive P in the P20 treatments; see Fig. 5). However, there was less total P and unreactive P in the plant-free controls, in comparison with the other treatments. Plant-available P comprised only a small portion of the total P in the soil (7.6% mean across all samples), and was significantly higher in P20 than in the P10 and P0 treatments.

Table 1
Statistical outcomes of two-way ANOVA for a range of plant physiological variables.

Plant biomass and P plant content	P treatment	Plant treatment (AM/non AM)	Interaction
SDW	<0.001	<0.001	ns
RDW	<0.001	0.001	ns
Shoot P content	<0.001	0.001	0.045
Root P content	<0.001	<0.001	ns

4. Discussion

To our knowledge, this is the first study to explore the influence of AMF on P leaching in a non-sterilized soil system, and the first to examine P composition and DOC concentration in the leachate. As expected, the amount of P leached was higher in soils with a higher starting P content. Intriguingly, however, increased P was leached where plants were present, and especially so when they were mycorrhizal, regardless of the greater plant biomass and P uptake in the mycorrhizal plants. Changes in the ratio of reactive to unreactive P in the leachates and a relationship between P leached and DOC suggest that the impacts of roots and AMF on P leaching may be more complex than a simple plant/fungal-mediated reduction in the pool of P available in the soil to be leached.

In our study, the use of the supplemental inoculation of *Rhizophagus irregularis* and the indigenous AMF community resulted an increase in plant biomass and tissue P concentration. This is consistent with a meta-analysis of 22 published studies which demonstrated that tissue P concentrations were generally higher in the 76R genotype than the *rmc* genotype in both root and shoot tissue (Watts-Williams and Cavagnaro, 2015).

By including the plant-free treatments in our study, we revealed the significant contribution of plant roots in term of leached volume, the total P and DOC content in the leachates. The leachate volume was greater from the plant-free treatments than those with plants, this might derive from effects of water absorption and exudation of plant roots. Plant root exudates such as mucilage, a polymeric gel, can increase the water holding capacity of soil (Kroener et al., 2016; Mimmo et al., 2003) especially in the rhizosphere (Kroener et al., 2014). Furthermore, the use of non-sterilized field soil here likely allowed the native soil microorganisms to produce compounds which can increase root cell permeability and thus increase the rate of root exudation (Barea et al., 2002).

Previous findings on the effect of AMF on P leaching vary across studies. AMF have been reported to reduce P leaching from soil (Asghari and Cavagnaro, 2011; van der Heijden, 2010; Bender et al., 2015), to have no effect on P leaching (Verbruggen et al., 2012; Köhl and van der Heijden, 2016), to both reduce and have no effect in the same experiment depending on soil P (Asghari et al., 2005), or to increase P leaching in some treatments (Bender and van der Heijden, 2015; Kohl et al., 2014). However, to our knowledge, our study is the first to show a significant and consistent increase in P leached with mycorrhizal plants (P10 and P20 treatments). Our inclusion of plant-free control treatments, coupled with a genotypic approach to control for the formation of AMF allow us to determine the effects of roots, both colonized by AMF and not, on P mobility with the native soil microbiome intact. Differences in leached total P were dominated by reactive P. Reactive P in the leachate accounted for 65–75% of total P, whereas the unreactive accounted for 25–35%. These proportions are similar to those reported for leachates from four grassland soil types using a large scale lysimeter in a field site (Turner and Haygarth, 2000).

The greater amount of reactive and total P leached in the presence of mycorrhizal root systems may be due to impact of AMF and other rhizosphere microorganisms as AMF can directly and indirectly impact soil microbial communities (Rillig, 2004). The activity of soil bacteria (Lecomte et al., 2011) and phosphate solubilising bacteria (Smith and Read, 2008; Toro et al., 1997), and phosphate solubility (Roy-Bolduc, 2011), have been found to be higher in the presence of AMF. Similarly, under P-deficient conditions, plant roots (Ryan et al., 2001) and soil rhizosphere microorganisms (Darch et al., 2016) can increase soil P by

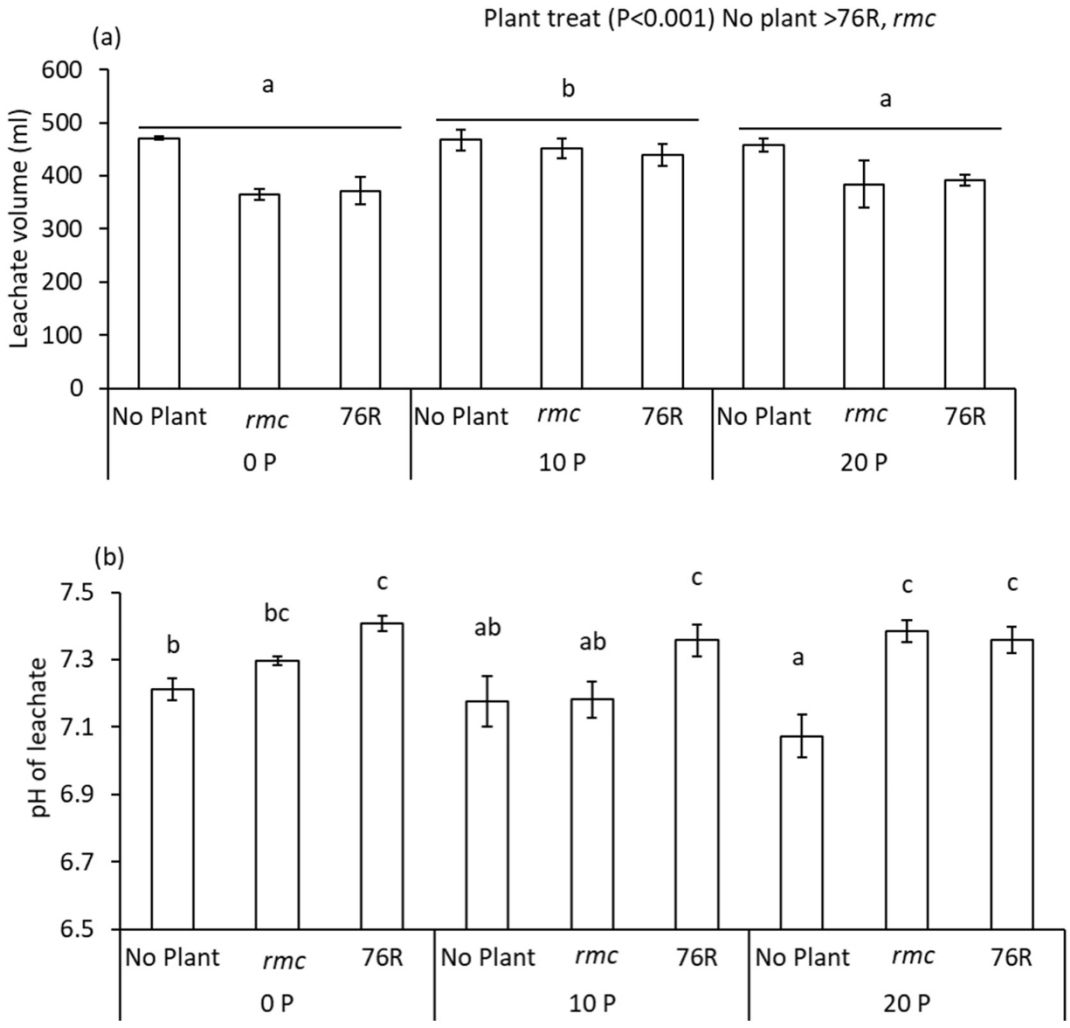

Fig. 2. Volume (a) and pH (b) of soil leachates. 76R and *rmc* are mycorrhizal and mycorrhiza-defective tomato genotypes. 0P, 10P and 20 P are P treatments at 0, 10 and 20 mg P kg^{-1} dry soil. "No plant" refers to plant-free treatments. Values are mean ± SE, n = 5. Means followed by the same letters are not significantly different at the P<0.05 level.

releasing organic anions and phosphatases to solubilize minerals and desorb P from minerals (Schachtman et al., 1998; Raghothama and Karthikeyan, 2005). Thus, the activities of plant roots and a range of soil microbes affect P mobility and enhance the probability of P loss through leaching (van der Heijden, 2010).

Although it is established that AMF can influence soil C cycling (Rillig, 2004), there is little information about the interaction of AMF and C leaching from soils. Our study is the first study to reveal the contribution of both plant roots and AMF to DOC leaching. Contrary to expectations, more P was leached from soils were mycorrhizal plants were present. This higher P in the leachate was coincident with differences in the colours of the leachates between treatments, prompting analysis of the DOC content of the leachates. This analysis revealed a strong correlation between total P leached and DOC. The increase in DOC for the plant treatments relative to the plant-free treatment can be attributed to root exudates. Exudation of organic compounds from plant roots is often associated with the release of polyvalent cations (Oades, 1984; Pojasok and Kay, 1990; Amezketa, 1999), which are known to participate in "bridging" interactions whereby the cations bind simultaneously to organic matter and phosphate (Xiong and Mahmood, 2010). Thus, simultaneous increases in the concentration of both DOC and polyvalent cations may be responsible for enhanced phosphate solubility and hence leaching. DOC is an important source of C for microbes (Hogberg and Hogberg, 2002); the greater amount of DOC leached in the mycorrhizal treatments may be associated with an increase in rhizosphere microbial activity that can increase production (GoEdde et al., 1996) and leaching of DOC (Christ and David, 1996). Alternatively, the presence of the polyvalent cations, which influence organic decomposition (Andersson et al., 2000), may have led to simultaneous P and DOC release from organic matter. Our study highlights the important potential contribution of AMF and soil microbial communities on P and DOC leached from soils, but there is a need for further study on these aspects.

Although the plant-free treatments had the highest leachate volume here, the total P and DOC leached and the reactive-unreactive P ratio was lowest and did not change with increased P addition. In contrast, within the plant treatments, the total P leached increased with P addition, leading to changes in the reactive-unreactive P ratio in the leachates of the plant treatments. Moreover, within the plant treatments, the total P and the DOC content in the leachates were higher in the mycorrhizal plant treatments than the non-mycorrhizal plant treatments in the P10 and P20 treatments. The P10 and P20 treatments were also where there was higher root biomass in the mycorrhizal plants than the non-mycorrhizal plants. It is possible that the greater plant root biomass observed was associated with the greater P and DOC found in the leachate of the mycorrhizal treatments, indicating a greater contribution by the roots and AMF to P and DOC leached. The leachate volume did not differ between AM and non-AM plants, which is consistent with earlier leaching experiments (Asghari and Cavagnaro, 2012; van der Heijden, 2010), suggesting that the difference in the mobility of P and DOC between mycorrhizal plant treatments and non-mycorrhizal plant treatments did not depend on the amount of water leached but

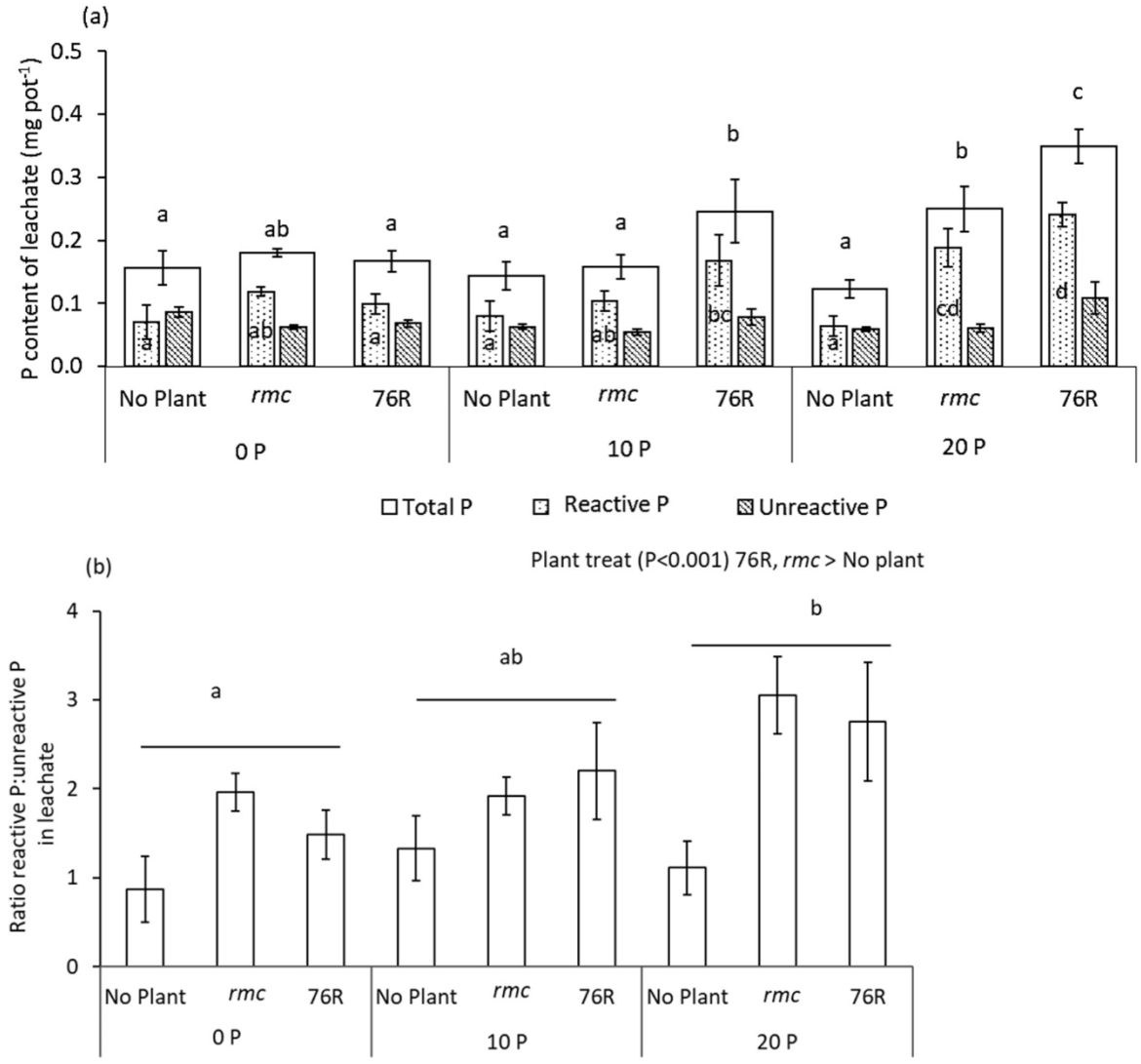

Fig. 3. Content of P leached from pots after leaching event. (a) Total dissolved P (white bars), reactive P, i.e. dissolved PO_4-P (dotted bars), "unreactive P", i.e. all P fractions besides dissolved PO_4-P (shaded bars) (b) Ratio of reactive P:unreactive P. 76R and *rmc* are mycorrhizal and mycorrhiza-defective tomato genotypes. 0P, 10P and 20 P are P treatments at 0, 10 and 20 mg P kg^{-1} dry soil. Values are mean ± SE, n = 5. Means followed by the same letter are not significantly different at the P<0.05 level.

possibly on the changes to soil P mobility and DOC in soils due to the association with AMF, soil microbial communities and plant roots.

After the leaching event, the plant-available P accounted for a small portion of the total P in soil, suggesting the addition at the rate of 10 and 20 mg kg^{-1} dry soil might not effectively elevate the plant P availability in soil. Moreover, the mean total P leached only accounted for 0.75% and 0.44% of P applied to the soil, and the total P contents of the soil (i.e., applied P + existing soil P), respectively. These findings likely reflect the relatively high P fixing capacity of the soil used, which was a loam containing 62.9% clay and silt.

Table 2
Statistical outcomes of two-way ANOVA for a range of leachate and soil physiological variables.

Leachate and soil	P treatment	Plant treatment (AM/non AM/no plant)	Interaction
Leachate volume	0.015	<0.001	ns
pH of leachate	ns	<0.001	0.028
Leachate total P	0.006	<0.001	0.005
Leachate reactive P	0.004	<0.001	0.027
Leachate unreactive P	ns	0.05	ns
Ratio of reactive P:unreactive P	0.04	0.001	ns
DOC content of leachate	<0.001	<0.001	0.002
Soil total P	<0.001	<0.05	ns
Soil reactive P	<0.001	ns	0.001
Soil unreactive P	<0.05	<0.001	ns

In summary, although AMF can have a positive impact on tomato P uptake and biomass, their influence on soil P leaching should be carefully evaluated as nutrient leaching is a major factor from a soil management perspective. In contrast to previous studies, we found an increase in P loss via leaching where plants formed arbuscular mycorrhizas, and when plants were present compared to when they were not. The increase in P loss may be associated with greater P mobility from soils, suggesting soil characteristic changes conferred by the roots of plants colonized by AMF. Such differences might relate to changes in microbial communities and plant root exudate chemistry variations between mycorrhizal and non-mycorrhizal plant roots. As these aspects were not included in our study, further work should focus on the changes of these factors under the mycorrhizal root systems to underline the effect of AMF on P soil loss.

Specifically, there was a great capacity for P uptake through both the root and AMF pathways, thus measures of available soil P may not be an adequate indicator of fertilizer application requirements in the field. This emphasises the potential role of plant roots for 'mining' P from the soil under P limitation conditions. The shift of reactive and unreactive P composition, and the strong correlation between total P and DOC in the leachates may underline

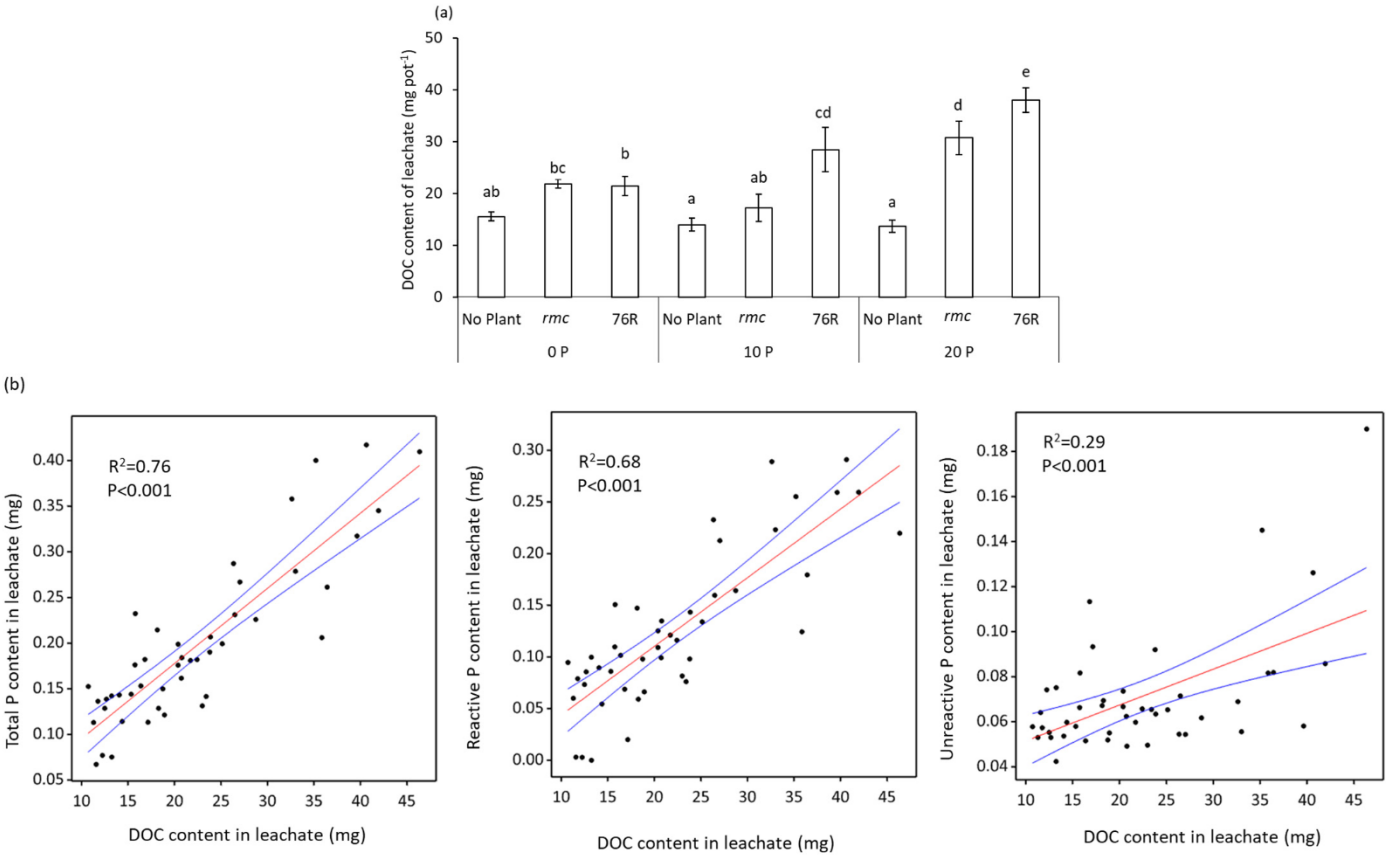

Fig. 4. (a) Dissolved organic content in the leachate (b) The regression between total P content and DOC content in the leachates. Values are mean ± SE, n = 5. Means followed by the same letter are not significantly different at the P<0.05 level. Red line: linear regression model; blue line: 95% confidence interval for model.

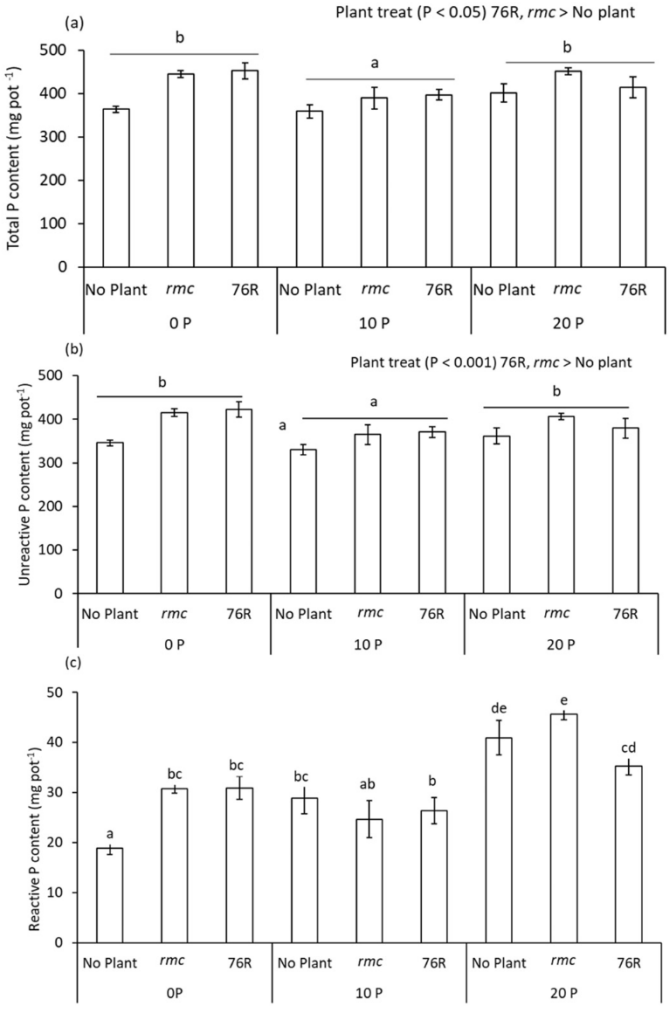

Fig. 5. Phosphorus content (mg pot^{-1}) in soil samples after the leaching event. (a) Total phosphorus, (b) "unreactive P", (c) reactive PO_4-P. 76R and *rmc* are mycorrhizal and mycorrhiza-defective tomato genotypes. 0P, 10P and 20 P are P treatments at 0, 10 and 20 mg P kg^{-1} dry soil. Values are mean ± SE, n = 5. Means followed by the same letter are not significantly different at the P<0.05 level.

these interesting results. By using the mycorrhiza-defective tomato as a tool, our study reveals new and counterintuitive findings that AMF not only enhance P uptake but might be associated with high P mobility in planted systems. This study also provides a further step in understanding the potential mechanism of the influence of AMF in P leaching, however, it also underscores the need for more studies to confirm the impacts of AMF and plant roots on soil biological, chemical and physical properties.

CRediT authorship contribution statement

Cuc T.K. Tran: Investigation, Writing - original draft, Formal analysis, Visualization. **Stephanie J. Watts-Williams:** Supervision, Validation, Writing - review & editing. **Ronald J. Smernik:** Supervision, Validation, Writing - review & editing. **Timothy R. Cavagnaro:** Supervision, Conceptualization, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Amezketa, E., 1999. Soil aggregate stability:a review. J. Sustain. Agric. 14 (2–3), 83–151. https://doi.org/10.1300/J064v14n02_08.
Andersson, S., Nilsson, S.I., Saetre, P., 2000. Leaching of dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) in mor humus as affected by temperature and pH. Soil Biol. Biochem. 32 (1), 1–10. https://doi.org/10.1016/S0038-0717(99)00103-0.
Asghari, H.R., Cavagnaro, T.R., 2011. Arbuscular mycorrhizas enhance plant interception of leached nutrients. Funct. Plant Biol. 38 (3), 219–226. https://doi.org/10.1071/FP10180.
Asghari, H.R., Cavagnaro, T.R., 2012. Arbuscular mycorrhizas reduce nitrogen loss via leaching. Ploss one 7 (1).
Asghari, H.R., Chittleborough, D.J., Smith, F.A., Smith, S.E., 2005. Influence of arbuscular mycorrhizal (AM) symbiosis on phosphorus leaching through soil cores. Plant Soil 275 (1), 181–193. https://doi.org/10.1007/s11104-005-1328-2.
Barea, J.-M., Azcón, R., Azcón-Aguilar, C., 2002. Mycorrhizosphere interactions to improve plant fitness and soil quality. Antonie Van Leeuwenhoek 81 (1), 343–351. https://doi.org/10.1023/a:1020588701325.
Barker, S.J., Stummer, B., Gao, L., Dispain, I., O'Connor, P.J., Smith, S.E., 1998. A mutant in Lycopersicon esculentum Mill. with highly reduced VA mycorrhizal colonization: isolation and preliminary characterisation. Plant J. 15 (6), 791–797. https://doi.org/10.1046/j.1365-313X.1998.00252.x.
Bender, S.F., van der Heijden, M.G.A., 2015. Soil biota enhance agricultural sustainability by improving crop yield, nutrient uptake and reducing nitrogen leaching losses. J. Appl. Ecol. 52 (1), 228–239. https://doi.org/10.1111/1365-2664.12351.
Bender, S.F., Conen, F., Van der Heijden, M.G.A., 2015. Mycorrhizal effects on nutrient cycling, nutrient leaching and N_2O production in experimental grassland. Soil Biol. Biochem. 80 (Supplement C), 283–292. https://doi.org/10.1016/j.soilbio.2014.10.016.
Bowles, T.M., Jackson, L.E., Cavagnaro, T.R., 2017. Mycorrhizal fungi enhance plant nutrient acquisition and module nitrogen loss with variable water regimes. Glob. Chang. Biol. https://doi.org/10.1111/gcb.13884.
Brookes, P.C., Heckrath, G., Smet, J.D., Hofman, G., Vanderdeelen, J., 1997. Losses of phosphorus indrainage water. In: Tunney, H., Carton, O.T., Brookes, P.C., Johnston, A.E. (Eds.), Phosphorus Loss From Soil to Water CAB International Wallingford, pp. 253–271.
Cavagnaro, T.R., 2008. The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: a review. Plant Soil 304 (1–2), 315–325. https://doi.org/10.1007/s11104-008-9559-7.
Cavagnaro, T.R., 2016. Soil moisture legacy effects: impacts on soil nutrients, plants and mycorrhizal responsiveness. Soil Biol. Biochem. 95, 173–179. https://doi.org/10.1016/j.soilbio.2015.12.016.
Cavagnaro, T.R., Smith, F.A., Lorimer, M.F., Haskard, K.A., Ayling, S.M., Smith, S.E., 2001. Quantitative development of Paris-type arbuscular mycorrhizas formed between Asphodelus fistulosus and Glomus coronatum. New Phytol. 149 (1), 105–113. https://doi.org/10.1046/j.1469-8137.2001.00001.x.
Cavagnaro, T.R., Bender, S.F., Asghari, H.R., van der Heijden, M.G.A., 2015. The role of arbuscular mycorrhizas in reducing soil nutrient loss. Trends Plant Sci. 20 (5), 283–290. https://doi.org/10.1016/j.tplants.2015.03.004.
Christ, M.J., David, M.B., 1996. Temperature and moisture effects on the production of dissolved organic carbon in a Spodosol. Soil Biol. Biochem. 28 (9), 1191–1199. https://doi.org/10.1016/0038-0717(96)00120-4.
Colwell, J.D., 1963. The estimation of phosphorus fertiliser requirements of wheat in southern. New South Wales Australian Journal of Experimental Agriculture 190–197.
Corkidi, L., Merhaut, D.J., Allen, E.B., Downer, J., Bohn, J., Evans, M., 2011. Effects of mycorrhizal colonization on nitrogen and phosphorus leaching from nursery containers. Effects of mycorrhizal colonization on nitrogen and phosphorus leaching from nursery containers 46 (11), 1472–1479. https://doi.org/10.21273/HORTSCI.46.11.1472.
Darch, T., Blackwell, M.S.A., Chadwick, D., Haygarth, P.M., Hawkins, J.M.B., Turner, B.L., 2016. Assessment of bioavailable organic phosphorus in tropical forest soils by organic acid extraction and phosphatase hydrolysis. Geoderma 284, 93–102. https://doi.org/10.1016/j.geoderma.2016.08.018.
Frossard, E., Condron, L.M., Oberson, A., Sinaj, S., Fardeau, J.C., 2000. Processes governing phosphorus availability in temperate soils. J. Environ. Qual. 29 (1), 15–23. https://doi.org/10.2134/jeq2000.00472425002900010003x.
Giovannetti, M., Mosse, B., 1980. Evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84, 498–500.
GoEdde, M., David, M.B., Christ, M.J., Kaupenjohann, M., Vance, G.F., 1996. Carbon mobilization from the forest floor under red spruce in the northeastern U.S.A. Soil Biol. Biochem. 28 (9), 1181–1189. https://doi.org/10.1016/0038-0717(96)00130-7.
van der Heijden, M.G.A., 2010. Mycorrhizal fungi reduce nutrient loss from model grassland ecosystems. Ecology 91 (4), 1163–1171. https://doi.org/10.1890/09-0336.1.
Hogberg, M.N., Hogberg, P., 2002. Extramatrical ectomycorrhizal mycelium contributes oneъЂђthird of microbial biomass and produces, together with associated roots, half the dissolved organic carbon in a forest soil. New Phytol. 154 (3), 791–795. https://doi.org/10.1046/j.1469-8137.2002.00417.x.

Köhl, L., van der Heijden, M.G.A., 2016. Arbuscular mycorrhizal fungal species differ in their effect on nutrient leaching. Soil Biol. Biochem. 94, 191–199. https://doi.org/10.1016/j.soilbio.2015.11.019.
Kohl, L., van der Heijden, M.G.A., Oehl, F., 2014. Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota. Report. Ecol. Appl. 24 (7).
Kroener, E., Zarebanadkouki, M., Kaestner, A., Carminati, A., 2014. Nonequilibrium water dynamics in the rhizosphere: how mucilage affects water flow in soils. Water Resour. Res. 50 (8), 6479–6495. https://doi.org/10.1002/2013WR014756.
Kroener, E., Zarebanadkouki, M., Bittelli, M., Carminati, A., 2016. Simulation of root water uptake under consideration of nonequilibrium dynamics in the rhizosphere. Water Resour. Res. 52 (8), 5755–5770. https://doi.org/10.1002/2015WR018579.
Lecomte, J., St-Arnaud, M., Hijri, M., 2011. Isolation and identification of soil bacteria growing at the expense of arbuscular mycorrhizal fungi. FEMS Microbiol. Lett. 317 (1), 43–51. https://doi.org/10.1111/j.1574-6968.2011.02209.x.
McLaughlin, M.J. FiRaTAR, 1991. Operation of the phosphorus, sulphur and nitrogen cycles. In: Barson, RmGaMM (Ed.), Australia's Renewable Resources : Sustainability and Global Change. Bureau of Rural Resources, Canbera, Autralia, pp. 67–116.
Marschner, H., Dell, B., 1994. Nutrient uptake in mycorrhizal symbiosis. Plant Soil 159 (1), 89–102. https://doi.org/10.1007/BF00000098.
Mimmo, T., Marzadori, C., Francioso, O., Deiana, S., Gessa, C.E., 2003. Effects of aluminum sorption on calciumвЂђpolygalacturonate network used as soilвЂђroot interface model. Biopolymers 70 (4), 655–661. https://doi.org/10.1002/bip.10545.
Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5.
Neumann, A., Torstensson, G., Aronsson, H., 2012. Nitrogen and phosphorus leaching losses from potatoes with diiferent harvest times and following crops. Field Crop Res. 133, 130–138.
Ngo, H.T.T., Cavagnaro, T.R., 2018. Interactive effects of compost and pre-planting soil moisture on plant biomass, nutrition and formation of mycorrhizas: a context dependent response. Sci. Rep. 8 (1), 1509. https://doi.org/10.1038/s41598-017-18780-2.
Oades, J., 1984. Soil organic matter and structural stability: mechanisms and implications for management. An International Journal on Plant-Soil Relationships 76 (1), 319–337. https://doi.org/10.1007/BF02205590.
Ozanne, P.G., Kirton, D.J., Shaw, T.C., 1961. The loss of phosphorus from sandy soils. Aust. J. Agric. Res. 12 (3), 409–423. https://doi.org/10.1071/AR9610409.
Pojasok, T., Kay, B.D., 1990. Effect of root exudates from corn and bromegrass on soil structural stability. Effect of root exudates from corn and bromegrass on soil structural stability 70 (3), 351–362. https://doi.org/10.4141/cjss90-036.
Pote, D., Daniel, T., Delaune, P., 2009. Total phosphorus and dissolved phosphorus in water samples. In: Kovar, J., Pierzynski, J. (Eds.), Methods of Phosphorus Analysis for Soils, Sediment, Residuals and Waters. Virginia tech University, Blacksburg, VA, USA, pp. 113–117.
Raghothama, K., Karthikeyan, A., 2005. Phosphate acquisition. An International Journal on Plant-Soil Relationships 274 (1), 37–49. https://doi.org/10.1007/s11104-004-2005-6.
Reid, K., Schneider, K., McConkey, B., 2018. Components of phosphorus loss from agricultural landscapes, and how to incorporate them into risk assessment tools. Front. Earth Sci. 6. https://doi.org/10.3389/feart.2018.00135.
Rillig, M.C., 2004. Arbuscular Mycorrhizae and Terrestrial Ecosystem Processes. vol 7. https://doi.org/10.1111/j.1461-0248.2004.00620.x Oxford, UK.
Rillig, M., Ramsey, P., Gannon, J., Mummey, D., Gadkar, V., Kapulnik, Y., 2008. Suitability of mycorrhiza-defective mutant/wildtype plant pairs (Solanum lycopersicum L. cv Micro-Tom) to address questions in mycorrhizal soil ecology. An International Journal on Plant-Soil Relationships 308 (1), 267–275. https://doi.org/10.1007/s11104-008-9629-x.
Roy-Bolduc, A.H.M., 2011. The use of mycorrhizae to enhance phosphorus uptake: a way out pf the phosphorus crisis. J Biofertil Biopestici 2, 104. https://doi.org/10.4172/2155-6202.1000104.
Ryan, P.R., Delhaize, E., Jones, D.L., 2001. Function and mechanism of organic anion exudation from plant roots. Annu. Rev. Plant Biol. 52, 527–560.
Schachtman, Reid, Ayling, 1998. Phosphorus uptake by plants: from soil to cell. Plant Physiol. 116 (2), 447. https://doi.org/10.1104/pp.116.2.447.
Sharpley, A.N., Rekolainen, S., 1997. Phosphorus in agriculture and its enviromental implications. In: Tunney, H., Carton, O.T., Brookes, P.C., Johnston, A.E. (Eds.), Phosphorus Loss From Soil to Water CAB International, Wallingford, pp. 1–53.
Smith, S.E., Read, D., 2008. Mycorrhizal Symbiosis. third ed. Academic Press, Elsevier Ltd., Oxford.
Smith, S.E., Smith, F.A., 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annu. Rev. Plant Biol. vol 62. https://doi.org/10.1146/annurev-arplant-042110-103846.
Toro, M., Azcon, R., Barea, J.M., 1997. Improvement of Arbuscular Mycorrhiza Development by Inoculation of Soil With Phosphate-solubilizing Rhizobacteria to Improve Rock Phosphate Bioavailability (32P) and Nutrient Cycling. 11 pp. 4408–4412.
Turner, B.L., Haygarth, P.M., 2000. Phosphorus forms and concentrations in leachate under four grassland soil types. Soil Sci. Soc. Am. J. 64 (3), 1090. https://doi.org/10.2136/sssaj2000.6431090x.
Turner, B.L., Paphzy, M.J., Haygarth, P.M., McKelvie, I.D., 2002. Inositol phosphates in the environment. Philosophical Transactions of the Royal Society B: Biological Sciences 357 (1420), 449–469. https://doi.org/10.1098/rstb.2001.0837.
Ulen, B., 1999. Leaching and balances of phosphorus and other nutrients in lysimeters after application of organic manures or fertilizers. Soil Use Manag. 15, 56–61.
Verbruggen, E., Kiers, E.T., Bakelaar, P.N.C., Röling, W.F.M., van der Heijden, M.G.A., 2012. Provision of contrasting ecosystem services by soil communities from different agricultural fields. Plant Soil 350 (1–2), 43–55. https://doi.org/10.1007/s11104-011-0828-5.
Vierheilig, H., Coughlan, A.P., Wyss, U., Piché, Y., 1998. Ink and vinegar, a simple staining technique for Arbuscular Mycorrhizal Fungi. Appl. Environ. Microbiol. 64 (12), 5004–5007.
Watts-Williams, S.J., Cavagnaro, T.R., 2014. Nutrient interactions and arbuscular mycorrhizas: a meta-analysis of a mycorrhiza-defective mutant and wild-type tomato genotype pair. Plant Soil 384 (1–2), 79–92. https://doi.org/10.1007/s11104-014-2140-7.
Watts-Williams, S.J., Cavagnaro, T.R., 2015. Using mycorrhiza-defective mutant genotypes of non-legume plant species to study the formation and functioning of arbuscular mycorrhiza: a review. Mycorrhiza 25 (8), 587–597. https://doi.org/10.1007/s00572-015-0639-2.
Wheal, M.S., Fowles, T.O., Palmer, L.T., 2011. A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of plant essential elements. Anal. Methods 3 (12), 2854–2863. https://doi.org/10.1039/C1AY05430A.
White, P.J., 2009. Efficiency of soil and fertilizer phosphorus use: reconciling changing concepts of soil phosphorus behaviour with agronomic information. Ex. Agric, 45 https://doi.org/10.1017/S0014479708007138.
Xiong, J., Mahmood, Q., 2010. Adsorptive removal of phosphate from aqueous media by peat. Desalination 259 (1–3), 59–64. https://doi.org/10.1016/j.desal.2010.04.035.

Chapter 3-Root and mycorrhizal effects on soil nutrient loss are modulated by soil texture

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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 lycopersicum* 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 soil 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.

1. 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 (Tran et al., 2020). This highlights the need for further information on the impacts of roots and AM on the leaching of P from soil in its various forms. Given the differences in the behaviour of P in different forms in the environment

Abbreviations: AM, Arbuscular mycorrhizas; AMF, Arbuscular mycorrhizal fungi; DOC, Dissolved organic carbon; P, Phosphorus; RDW, Root dry weight; *rmc*, Mycorrhiza-defective tomato mutant; 76R, Mycorrhizal wild type progenitor tomato; RO, Reverse osmosis; SDW, Shoot dry weight.

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(Toor et al., 2005), it is important to quantify not only the total amount of P leached, but also its chemical nature (e.g. reactive and unreactive) both in the leachate and the soil.

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

Soil P loss via leaching is complex and is affected by many edaphic factors, including chemical, hydrological (soil permeability, soil aggregation) (Maguire and Sims, 2002), and P-sorption properties (Djodjic et al., 2004). Leaching of P is particularly problematic in sandy soils where low P sorption capacity and relatively high hydraulic conductivity (Sims et al., 1998; Nelson et al., 2005) can lead to significant P loss during rainfall events. Despite this, to our knowledge, very few studies focused on the effect of AM on P leaching in a sandy soil. Moreover, in our previous leaching experiment, the mean total P leached only accounted for 0.75% of 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, there are relatively few studies of root and AM effects on the amount and nature of P remaining in the soil. To further explore this issue, there is a need to investigate impacts of roots and AM on soil P leaching in soils with contrasting textures.

Here we compared 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.

2. Materials and methods

2.1. 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 × 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.

2.2. 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 ($\Psi_m = -10$ 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 reverse osmosis (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 $K_2HPO_4{\cdot}3H_2O$ dissolved in 50 mL of 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 colonization (named *rmc* hereafter), and its mycorrhizal wild-type progenitor (named 76R hereafter) (Barker et al., 1998). This approach avoids the need to sterilise soil and thus ensures a natural soil microbiome is present for both genotypes (Rillig et al., 2008).

Seeds of the 76R and *rmc* tomato genotypes were shaken in a 10% sodium hypochlorite solution for 3 min to surface-sterilise the seeds. The seeds were then rinsed with RO water, and sown into coarse sand for germination. The seedlings with fully expanded cotyledons were transplanted into the microcosms (one seedling per microcosm) after one week.

2.3. Growth conditions

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

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

2.4. 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 each microcosm, simulating a rainfall event of 110 mm (Asghari and Cavagnaro, 2012). After 48 h, 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) and 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).

2.5. 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 h to determine the gravimetric water content. The remaining soils for P pool analysis were dried at 40 °C in the oven for 24 h.

The concentration of plant-available (Colwell) P in soil samples was determined using colorimetric assay (Murphy and Riley, 1962). The soil samples were extracted in 0.5 M sodium bicarbonate ($NaHCO_3$) solution at a soil:extractant ratio of 1:100 followed by 16 h 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 roots was stored in 70% 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 °C for 10 min (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 h, before root dry weight (RDW) and shoot dry weight (SDW) were 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).

2.6. 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 $\alpha < 0.05$).

3. Results

3.1. 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 (Fig. 1). Specifically, roots of the 76R plants grown in the coarse substrate, had a higher percent root length colonized in the lower soil layers than in the surface. In the fine substrate, colonization 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 (Fig. 2a). While there was no difference in the RDW between the two soil substrates, there was significantly greater SDW in the fine substrate compared to the coarse substrate ($P < 0.001$).

There was no difference in RDW between mycorrhizal and non-mycorrhizal roots between the three soil layers or two soil substrates (Fig. 2c). The top layer (0–10 cm) had the highest root biomass in both soil substrates. The roots in the sandier soil mix had greater biomass in the topsoil (0–10 cm) but lower in the bottom layer (25–35 cm) in comparison with root biomass 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 ($P < 0.01$) (Fig. 2b).

3.2. Leachate volume and nutrient content

After 48 h, 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 mean 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 mean 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 (Fig. 3). Additionally, whereas there was no difference in the leachate

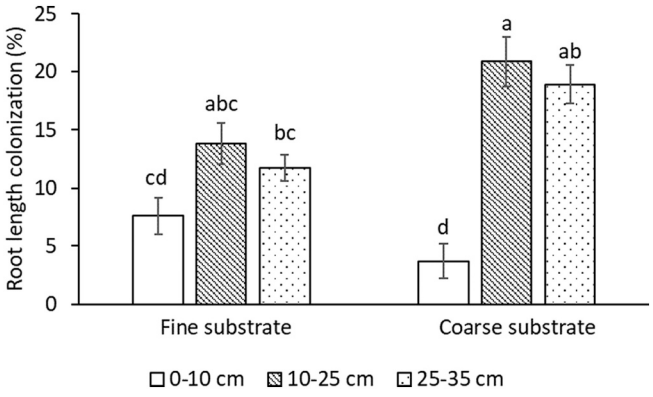

Fig. 1. Root length colonization of mycorrhizal plants (76R). Values are mean ± SEM, n = 5. Means followed by the same letters are not significantly different (Tukey's HSD; $\alpha = 0.05$).

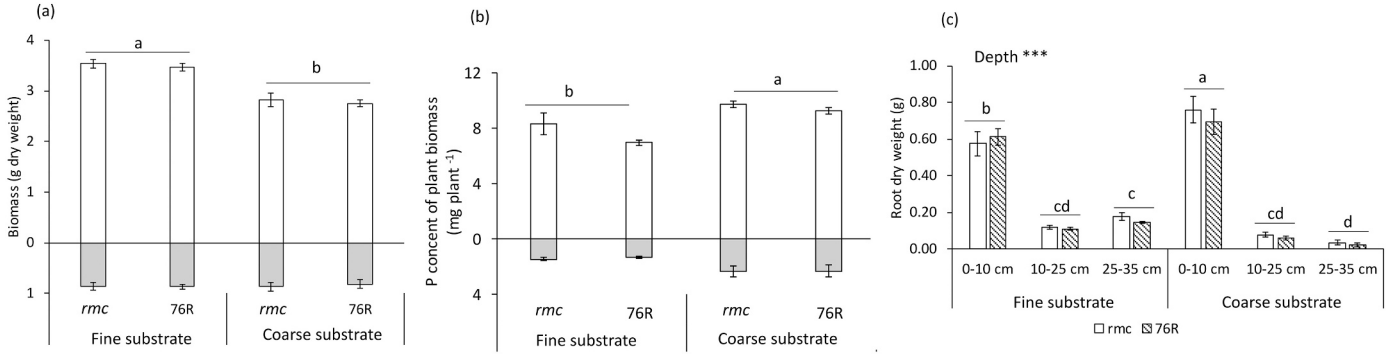

Fig. 2. Mean shoot (above x-axis) and root (below x-axis) dry weight (a) and plant P content of the mycorrhizal plant (76R) and mycorrhiza-defective tomato genotypes (*rmc*) (b) and the root biomass distribution at different soil depths and in two soil mixtures (c). Values are mean ± SEM, n = 5. Means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

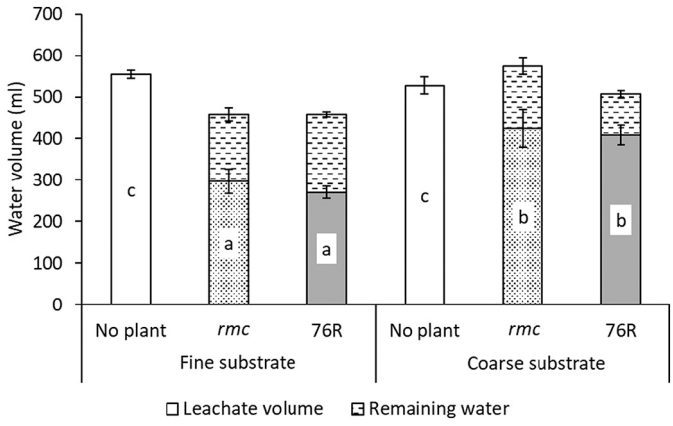

Fig. 3. Leachate volume and water remaining on the soil surface after leaching event (mL). N.B. there was no water remaining on the soil surface at the end of the leaching event in the no-plant treatment. 76R and *rmc* are mycorrhizal and mycorrhiza-defective tomato genotypes; "No plant" refers to plant-free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

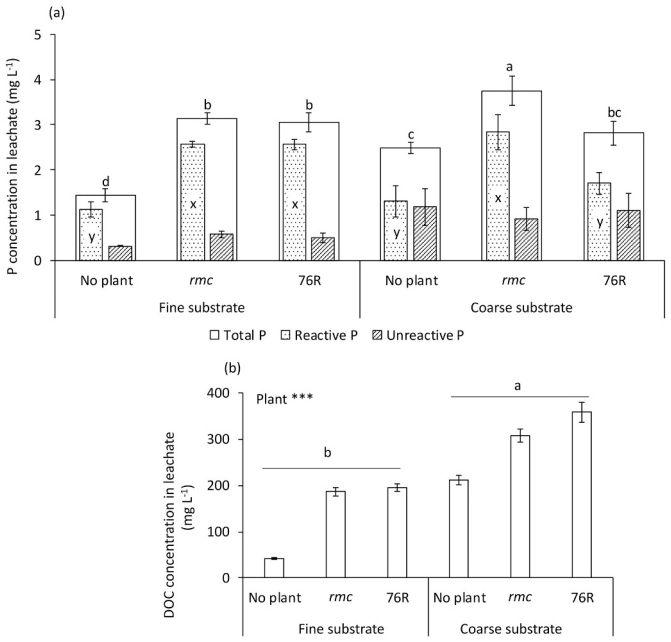

Fig. 4. Phosphorus (a) and dissolved organic carbon (b) concentration of soil leachate. 76R and *rmc* are mycorrhizal and mycorrhiza-defective tomato genotypes; "No plant" refers to plant-free treatments. Values are mean ± SEM, n = 5. Means followed by the same letters are not significantly different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

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

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

3.3. Soil moisture and soil P

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

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

Similar to the total soil P concentration, reactive soil P concentration of the fine 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

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

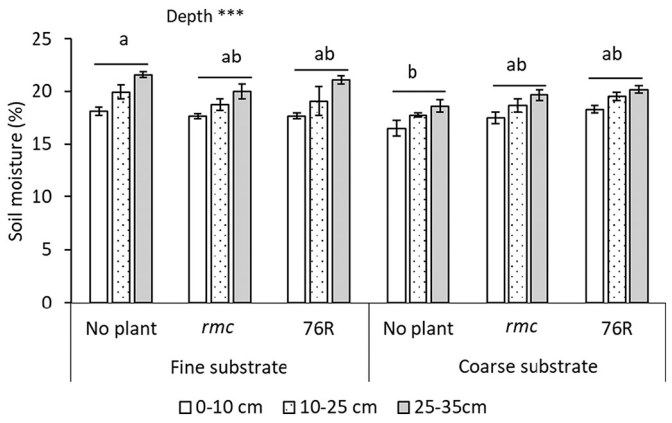

Fig. 5. Gravimetric water content (%) of soils, following soil depth after leaching event. 76R and *rmc* are mycorrhizal and mycorrhiza-defective tomato genotypes; "No plant" refers to plant-free treatments. Values are mean ± SEM, n = 5. Means followed by the same letters are not significantly different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

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.

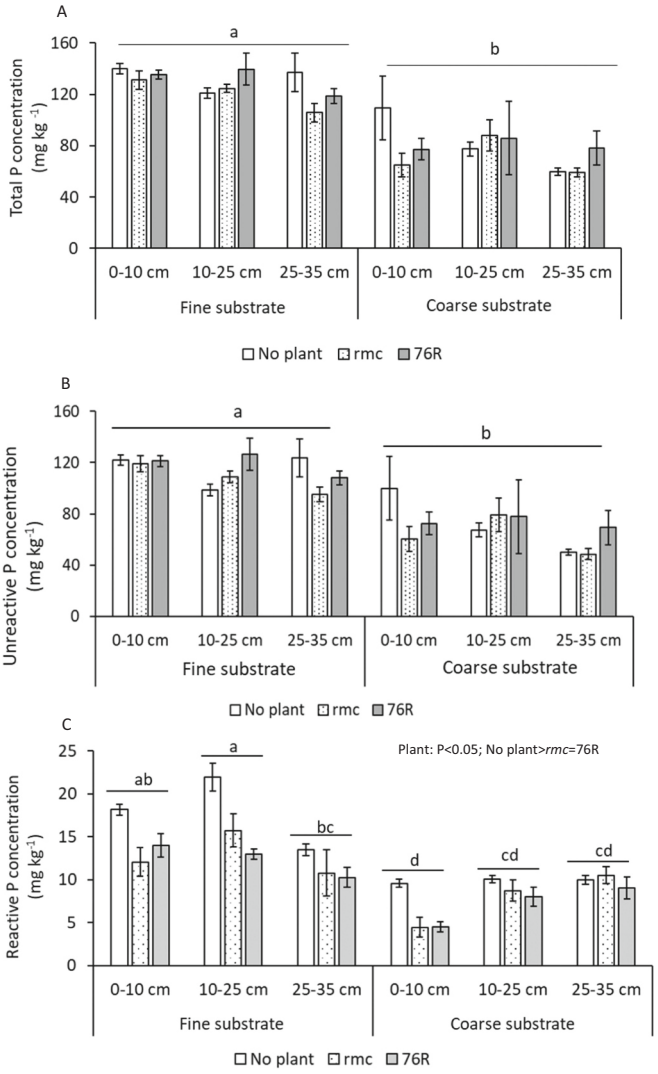

Fig. 6. Phosphorus concentration (mg kg^{-1}) in soil samples, after the leaching event, following soil depth. (A) Total phosphorus, (B) reactive phosphorus, (C) unreactive phosphorus. 76R and *rmc* are mycorrhizal and mycorrhiza-defective tomato genotypes; "No plant" refers to plant-free treatments.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

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

4. 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 mycorrhizal colonisation. Importantly, reactive P fractions are not only a directly available P source for plants but can also 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 leached. 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 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 were stimulated; this is, however, speculative and is worthy of further investigation. Also, the increase in soil microbial activities might enhance DOC production and leaching (Brooks et al., 1999; Christ and David, 1996).

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

A lower volume was leached from microcosms containing plants, with substantial amounts of water retained on the soil surface after 48 h. The presence of roots could lower the infiltration rates and hydraulic conductivity compared to unplanted soil (Leung et al., 2015) because roots have the capacity to block water flow channels created by soil pore spaces (Buczko et al., 2007; Scanlan, 2010). Another possible explanation is that root exudation might contribute to changes in the soil structure (Grayston et al., 1997; Traoré et al., 2000) and thus soil pore size, which may reduce soil infiltration rate and hydraulic conductivity. Although plant treatments had a lower leachate volume, concentration of DOC and P in leachate of these treatments were consistently higher than for plant-free treatments for both soil substrates. This can be explained by the contribution of root exudation (Nowack et al., 2008; Boddy et al., 2007) and rhizosphere microbial activity (by using non-sterilised soil substrate) (GoEdde et al., 1996) that would increase soil DOC. Also, DOC can interact with many soil chemicals, affecting their fate in soil (Fernández-Pérez et al., 2005). The presence of the DOC may decrease P adsorption (Kang et al., 2011) because of the competition of organic anions with P for sorption sites (Bhatti et al., 1998; Iyamuremye et al., 1996) or increase the negative charge on soil surfaces that can inhibit P adsorption (Barrow, 1989; Jiao et al., 2007). The interaction of P with DOC has also been reported to increase the mobility of soil P (Zsolnay and Görlitz, 1994; Alvarez et al., 2004). Taken together, these results highlight that root and AM impacts on soil P loss via leaching are more complex that a simple case of plant/AM P assimilation.

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

In summary, the results of this study show the different effects of AM on P leaching loss in two soil substrates differing in texture. This study also highlights the contribution of soil texture effects to 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 communities on nutrient cycling and leaching. It should be noted that the present study only included a single simulated

rainfall event under greenhouse conditions; it will be important to investigate effects of AM on P and nutrient soil loss under field conditions with natural rainfall or field irrigation. 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.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2021.104097.

References

Alvarez, R., Evans, L.A., Milham, P.J., Wilson, M.A., 2004. Effects of humic material on the precipitation of calcium phosphate. Geoderma 118 (3), 245–260. https://doi.org/10.1016/S0016-7061(03)00207-6.

Asghari, H.R., Cavagnaro, T.R., 2012. Arbuscular mycorrhizas reduce nitrogen loss via leaching. PLoS One 7 (1).

Asghari, H.R., Chittleborough, D.J., Smith, F.A., Smith, S.E., 2005. Influence of arbuscular mycorrhizal (AM) symbiosis on phosphorus leaching through soil cores. Plant Soil 275 (1), 181–193. https://doi.org/10.1007/s11104-005-1328-2.

Barker, S.J., Stummer, B., Gao, L., Dispain, I., O'Connor, P.J., Smith, S.E., 1998. A mutant in Lycopersicon esculentum Mill. with highly reduced VA mycorrhizal colonization: isolation and preliminary characterisation. Plant J. 15 (6), 791–797. https://doi.org/10.1046/j.1365-313X.1998.00252.x.

Barrow, N.J., 1989. Testing a mechanistic model. IX. Competition between anions for sorption by soil. J. Soil Sci. 40 (2), 415–425. https://doi.org/10.1111/j.1365-2389.1989.tb01284.x.

Bender, S.F., van der Heijden, M.G.A., 2015. Soil biota enhance agricultural sustainability by improving crop yield, nutrient uptake and reducing nitrogen leaching losses. J. Appl. Ecol. 52 (1), 228–239. https://doi.org/10.1111/1365-2664.12351.

Bender, S.F., Conen, F., Van der Heijden, M.G.A., 2014. Mycorrhizal effects on nutrient cycling, nutrient leaching and N2O production in experimental grassland. Soil Biol. Biochem. 80 (Supplement C):283-292 https://doi.org/10.1016/j.soilbio.2014.10.016.

Bhatti, J.S., Comerford, N.B., Johnston, C.T., 1998. Influence of oxalate and soil organic matter on sorption and desorption of phosphate onto a spodic horizon. Soil Sci. Soc. Am. J. (4), 1089–1095.

Boddy, E., Hill, P.W., Farrar, J., Jones, D.L., 2007. Fast turnover of low molecular weight components of the dissolved organic carbon pool of temperate grassland field soils. Soil Biol. Biochem. 39, 827–835.

Boesch, D.F., Brinsfield, R.B., Magnien, R.E., 2001. Chesapeake Bay eutrophication: scientific understanding, ecosystem restoration, and challenges for agriculture. J. Environ. Qual. 30 (2), 303–320. https://doi.org/10.2134/jeq2001.302303x.

Bowles, T.M., Barrios-Masias, F.H., Carlisle, E.A., Cavagnaro, T.R., Jackson, L.E., 2016. Effects of arbuscular mycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. Sci. Total Environ. 566-567, 1223–1234. https://doi.org/10.1016/j.scitotenv.2016.05.178.

Bowles, T.M., Jackson, L.E., Cavagnaro, T.R., 2017. Mycorrhizal fungi enhance plant nutrient acquisition and module nitrogen loss with variable water regimes. Glob. Chang. Biol. https://doi.org/10.1111/gcb.13884.

Brooks, P.D., McKnight, D.M., Bencala, K.E., 1999. The relationship between soil heterotrophic activity, soil dissolved organic carbon (DOC) leachate, and catchment-scale DOC export in headwater catchments. Water Resour. Res. 35 (6), 1895–1902. https://doi.org/10.1029/1998WR900125.

Brzostek, E., Greco, A., Drake, J., Finzi, A., 2013. Root carbon inputs to the rhizosphere stimulate extracellular enzyme activity and increase nitrogen availability in temperate forest soils. Biogeochemistry 115 (1–3), 65–76. https://doi.org/10.1007/s10533-012-9818-9.

Buczko, U., Bens, O., Hüttl, R.F., 2007. Changes in soil water repellency in a pine–beech forest transformation chronosequence: influence of antecedent rainfall and air temperatures. Ecol. Eng. 31 (3), 154–164. https://doi.org/10.1016/j.ecoleng.2007.03.006.

Cavagnaro, T.R., 2016. Soil moisture legacy effects: impacts on soil nutrients, plants and mycorrhizal responsiveness. Soil Biol. Biochem. 95, 173–179. https://doi.org/10.1016/j.soilbio.2015.12.016.

Cavagnaro, T.R., Smith, F.A., Lorimer, M.F., Haskard, K.A., Ayling, S.M., Smith, S.E., 2001. Quantitative development of Paris-type arbuscular mycorrhizas formed between Asphodelus fistulosus and Glomus coronatum. New Phytol. 149 (1), 105–113. https://doi.org/10.1046/j.1469-8137.2001.00001.x.

Cavagnaro, T.R., Jackson, L.E., Six, J., Ferris, H., Goyal, S., Asami, D., Scow, K.M., 2006. Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. Plant Soil 282 (1), 209–225. https://doi.org/10.1007/s11104-005-5847-7.

Cavagnaro, T.R., Bender, S.F., Asghari, H.R., van der Heijden, M.G.A., 2015. The role of arbuscular mycorrhizas in reducing soil nutrient loss. Trends Plant Sci. 20 (5), 283–290. https://doi.org/10.1016/j.tplants.2015.03.004.

Christ, M.J., David, M.B., 1996. Temperature and moisture effects on the production of dissolved organic carbon in a Spodosol. Soil Biol. Biochem. 28 (9), 1191–1199. https://doi.org/10.1016/0038-0717(96)00120-4.

Colwell, J.D., 1963. The estimation of phosphorus fertiliser requirements of wheat in southern New South Wales. Aust. J. Exp. Agric. 190–197.

Corkidi, L., Merhaut, D.J., Allen, E.B., Downer, J., Bohn, J., Evans, M., 2011. Effects of mycorrhizal colonization on nitrogen and phosphorus leaching from nursery containers. HortScience 46 (11), 1472–1479.

Djodjic, F., Börling, K., Bergström, L., 2004. Phosphorus leaching in relation to soil type and soil phosphorus content. J. Environ. Qual. 33 (2), 678–684. https://doi.org/10.2134/jeq2004.6780.

Duffková, R., Fučík, P., Jurkovská, L., Janoušková, M., 2019. Experimental evaluation of the potential of arbuscular mycorrhiza to modify nutrient leaching in three arable soils located on one slope. Appl. Soil Ecol. 143, 116–125. https://doi.org/10.1016/j.apsoil.2019.06.001.

Fernández-Pérez, M., Flores-Céspedes, F., González-Pradas, E., Ureña-Amate, M.D., Villafranca-Sánchez, M., Socías-Viciana, M., Pérez-García, S., 2005. Effects of dissolved organic carbon on phosphate retention on two calcareous soils. J. Agric. Food Chem. 53 (1), 84. https://doi.org/10.1021/jf048767o.

Giovannetti, M., Mosse, B., 1980. Evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytol. 84, 498–500.

GoEdde, M., David, M.B., Christ, M.J., Kaupenjohann, M., Vance, G.F., 1996. Carbon mobilization from the forest floor under red spruce in the northeastern U.S.A. Soil Biol. Biochem. 28 (9), 1181–1189. https://doi.org/10.1016/0038-0717(96)00130-7.

Grayston, S.J., Vaughan, D., Jones, D., 1997. Rhizosphere carbon flow in trees, in comparison with annual plants: the importance of root exudation and its impact on microbial activity and nutrient availability. Appl. Soil Ecol. 5 (1), 29–56. https://doi.org/10.1016/S0929-1393(96)00126-6.

Heckrath, G., Brookes, P.C., Poulton, P.R., Goulding, K.W.T., 1995. Phosphorus leaching from soils containing different phosphorus concentrations in the Broadbalk experiment. J. Environ. Qual. 24 (5), 904–910. https://doi.org/10.2134/jeq1995.00472425002400050018x.

Houben, D., Sonnet, P., 2012. Zinc mineral weathering as affected by plant roots. Appl. Geochem. 27 (8), 1587. https://doi.org/10.1016/j.apgeochem.2012.05.004.

Iyamuremye, F., Dick, R.P., Baham, J., 1996. Organic amendments and phosphorus dynamics. I. Phosphorus chemistry and sorption. Soil Sci. 161 (7), 426–435. https://doi.org/10.1097/00010694-199607000-00002.

Jaitz, L., Mueller, B., Koellensperger, G., Huber, D., Oburger, E., Puschenreiter, M., Hann, S., 2011. LC-MS analysis of low molecular weight organic acids derived from root exudation. Anal. Bioanal. Chem. 400 (8), 2587. https://doi.org/10.1007/s00216-010-4090-0.

Jakobsen, I., Abbott, L.K., Robson, A.D., 1992. External hyphae of vesicular—arbuscular mycorrhizal fungi associated with Trifolium subterraneum L. New Phytol. 120 (4), 509–516. https://doi.org/10.1111/j.1469-8137.1992.tb01800.x.

Jansa, J., Mozafar, A., Frossard, E., 2005. Phosphorus acquisition strategies within arbuscular mycorrhizal fungal community of a single field site. Plant Soil 276 (1), 163–176. https://doi.org/10.1007/s11104-005-4274-0.

Jiao, Y., Whalen, J.K., Hendershot, W.H., 2007. Phosphate sorption and release in a sandy-loam soil as influenced by fertilizer sources. Soil Sci. Soc. Am. J. 71 (1), 118–124. https://doi.org/10.2136/sssaj2006.0028.

Junguo, L., Liangzhi, Y., Manouchehr, A., Michael, O., Mario, H., Alexander, J.B.Z., Hong, Y., 2010. A high-resolution assessment on global nitrogen flows in cropland. Proc. Natl. Acad. Sci. 107 (17), 8035. https://doi.org/10.1073/pnas.0913658107.

Kang, J., Amoozegar, A., Hesterberg, D., Osmond, D.L., 2011. Phosphorus leaching in a sandy soil as affected by organic and inorganic fertilizer sources. Geoderma 161 (3), 194–201. https://doi.org/10.1016/j.geoderma.2010.12.019.

Köhl, L., van der Heijden, M.G.A., 2016. Arbuscular mycorrhizal fungal species differ in their effect on nutrient leaching. Soil Biol. Biochem. 94, 191–199. https://doi.org/10.1016/j.soilbio.2015.11.019.

Leung, A.K., Garg, A., Coo, J.L., Ng, C.W.W., Hau, B.C.H., 2015. Effects of the roots of Cynodon dactylon and Schefflera heptaphylla on water infiltration rate and soil hydraulic conductivity:effects of plant roots on infiltration characteristics and suction. Hydrol. Process. 29 (15), 3342–3354. https://doi.org/10.1002/hyp.10452.

Maguire, R.O., Sims, J.T., 2002. Soil testing to predict phosphorus leaching published as paper no. 1710 in the journal series of the Delaware Agricultural Experiment Station. J. Environ. Qual. 31 (5), 1601–1609. https://doi.org/10.2134/jeq2002.1601.

Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5.

Nannipieri, P., Ascher, J., Ceccherini, M.T., Pietramellara, G., Renella, G., Valori, F., 2008. Effects of Root Exudates in Microbial Diversity and Activity in Rhizosphere Soils, Vol 15. Soil Biology. Springer, Berlin Heidelberg, Berlin, Heidelberg. https://doi.org/10.1007/978-3-540-75575-3_14.

Nelson, N.O., Parsons, J.E., Mikkelsen, R.L., 2005. Field-scale evaluation of phosphorus leaching in acid sandy soils receiving swine waste. J. Environ. Qual. 34 (6), 2024–2035. https://doi.org/10.2134/jeq2004.0445.

Neumann G RV (2007) The release of root exudates as affected by the plant physiological status. In: Pinton R VZ, Nannipieri P (ed) The Rhizosphere: Biochemistry and Organic Substances at the Soil–Plant Interface. 2nd edn. CRC, Boca Raton.

Nowack, B., Schulin, R., Tercier-Waeber, M.L., Luster, J., 2008. Metal solubility and speciation in the rhizosphere of Lupinus albus cluster roots. Environ. Sci. Technol. 42 (19), 7146. https://doi.org/10.1021/es800167g.

Parihar, M., Meena, V., Mishra, P., Rakshit, A., Choudhary, M., Yadav, R., Rana, K., Bisht, J., 2019. Arbuscular mycorrhiza: a viable strategy for soil nutrient loss reduction. Arch. Microbiol. 201 (6), 723–735. https://doi.org/10.1007/s00203-019-01653-9.

R Core Team, 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL. https://www.R-project.org/.

Rillig, M., Ramsey, P., Gannon, J., Mummey, D., Gadkar, V., Kapulnik, Y., 2008. Suitability of mycorrhiza-defective mutant/wildtype plant pairs (Solanum lycopersicum L. cv Micro-Tom) to address questions in mycorrhizal soil ecology. Plant Soil 308 (1), 267–275. https://doi.org/10.1007/s11104-008-9629-x.

Rodríguez-Echeverría, S., Freitas, H., 2006. Diversity of AMF associated with Ammophila arenaria ssp. arundinacea in Portuguese sand dunes. Mycorrhiza 16 (8), 543–552. https://doi.org/10.1007/s00572-006-0070-9.

Scanlan, C.H.C., 2010. Insights into the processes and effects of root-induced changes to soil hydraulic properties. In: Proceeding of the 19th World Congress of Soil Science 2 (Brisbane, Australia), pp. 41–44.

Sharpley, A.N., Rekolainen, S., 1997. Phosphorus in agriculture and its enviromental implications In. In: Tunney, H., Carton, O.T., Brookes, P.C., Johnston, A.E. (Eds.), Phosphorus Loss From Soil to Water CAB International, Wallingford, pp. 1–53.

Sims, J.T., Simard, R.R., Joern, B.C., 1998. Phosphorus loss in agricultural drainage: historical perspective and current research. J. Environ. Qual. (2), 277–293.

Smith, S.E., Smith, F.A., 2011. Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annu. Rev. Plant Biol. 62 https://doi.org/10.1146/annurev-arplant-042110-103846.

Springmann, M., Clark, M., Mason-D'Croz, D., Wiebe, K., Bodirsky, B.L., Lassaletta, L., de Vries, W., Vermeulen, S.J., Herrero, M., Carlson, K.M., Jonell, M., Troell, M., Declerck, F., Gordon, L.J., Zurayk, R., Scarborough, P., Rayner, M., Loken, B., Fanzo, J., HCJ, Godfray, Tilman, D., Rockström, J., Willett, W., 2018. Options for keeping the food system within environmental limits. Nature 562 (7728), 519. https://doi.org/10.1038/s41586-018-0594-0.

Toor, G.S., Condron, L.M., Cade-Menun, B.J., Di, H.J., Cameron, K.C., 2005. Preferential phosphorus leaching from an irrigated grassland soil. Eur. J. Soil Sci. 56 (2), 155–168. https://doi.org/10.1111/j.1365-2389.2004.00656.x.

Tran, C.T.K., Watts-Williams, S.J., Smernik, R.J., Cavagnaro, T.R., 2020. Effects of plant roots and arbuscular mycorrhizas on soil phosphorus leaching. Sci. Total Environ. 722 https://doi.org/10.1016/j.scitotenv.2020.137847.

Traoré, O., Groleau-Renaud, V., Plantureux, S., Tubeileh, A., Boeuf-Tremblay, V., 2000. Effect of root mucilage and modelled root exudates on soil structure. Eur. J. Soil Sci. 51 (4), 575–581. https://doi.org/10.1111/j.1365-2389.2000.00348.x.

Turner, B.L., Haygarth, P.M., 2000. Phosphorus forms and concentrations in leachate under four grassland soil types. Soil Sci. Soc. Am. J. 64 (3), 1090. https://doi.org/10.2136/sssaj2000.6431090x.

van der Heijden, M.G.A., 2010. Mycorrhizal fungi reduce nutrient loss from model grassland ecosystems. Ecology 91 (4), 1163–1171. https://doi.org/10.1890/09-0336.1.

Verbruggen, E., Kiers, E.T., Bakelaar, P.N.C., Röling, W.F.M., van der Heijden, M.G.A., 2012. Provision of contrasting ecosystem services by soil communities from different agricultural fields. Plant Soil 350 (1–2), 43–55. https://doi.org/10.1007/s11104-011-0828-5.

Vierheilig, H., Coughlan, A.P., Wyss, U., Piché, Y., 1998. Ink and vinegar, a simple staining technique for arbuscular mycorrhizal fungi. Appl. Environ. Microbiol. 64 (12), 5004–5007.

Weaver, D., Ritchie, G., Anderson, G., Deeley, D., 1988. Phosphorus leaching in sandy soils. I. Short-term effects of fertilizer applications and environmental conditions. Aust. J. Soil Res. 26 (1), 177. https://doi.org/10.1071/SR9880177.

Wheal, M.S., Fowles, T.O., Palmer, L.T., 2011. A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of plant essential elements. Anal. Methods 3 (12), 2854–2863. https://doi.org/10.1039/C1AY05430A.

Xiao, L., Bi, Y., Du, S., Wang, Y., Guo, C., 2019. Effects of re-vegetation type and arbuscular mycorrhizal fungal inoculation on soil enzyme activities and microbial biomass in coal mining subsidence areas of Northern China. Catena 177, 202–209. https://doi.org/10.1016/j.catena.2019.02.019.

Zaller, J.G., Frank, T., Drapela, T., 2011. Soil sand content can alter effects of different taxa of mycorrhizal fungi on plant biomass production of grassland species. Eur. J. Soil Biol. 47 (3), 175–181. https://doi.org/10.1016/j.ejsobi.2011.03.001.

Zarea, M.J., Ghalavand, A., Goltapeh, E.M., Rejali, F., Zamaniyan, M., 2009. Effects of mixed cropping, earthworms (Pheretima sp.), and arbuscular mycorrhizal fungi (Glomus mosseae) on plant yield, mycorrhizal colonization rate, soil microbial biomass, and nitrogenase activity of free-living rhizosphere bacteria. Pedobiologia 52 (4), 223–235. https://doi.org/10.1016/j.pedobi.2008.10.004.

Zhang, S., Yu, X., Rillig, M., 2020. Arbuscular mycorrhiza contributes to the control of phosphorus loss in paddy fields. Plant Soil 447 (1–2), 623–636. https://doi.org/10.1007/s11104-019-04394-2.

Zsolnay, A., Görlitz, H., 1994. Water extractable organic matter in arable soils: effects of drought and long-term fertilization. Soil Biol. Biochem. 26 (9), 1257–1261. https://doi.org/10.1016/0038-0717(94)90151-1.

Supplementary data

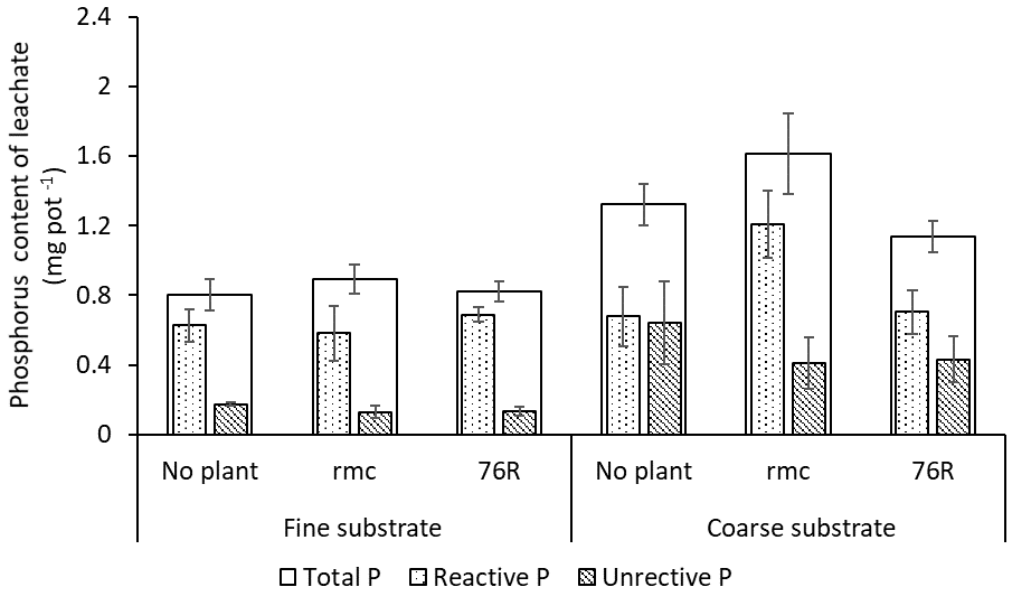

Figure S1. Phosphorus content of soil leachate. 76R and *rmc* are mycorrhizal and mycorrhiza-defective tomato genotypes; "No plant" refers to plant-free treatments. Values are mean ± SEM, n = 5.

Chapter 4-Arbuscular mycorrhizas and field-grown tomatoes: a study of growth, phosphorus leaching, soil moisture and the wider soil microbial community.

This work contained in this chapter has been prepared for submission to *Science of the Total Environment* and is presented here in the journal format.

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Arbuscular mycorrhizas and field-grown tomatoes: a study of growth, phosphorus leaching, soil moisture and the wider soil microbial community.

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Abstract

While interest in arbuscular mycorrhizal (AM) fungal effects on soil phosphorus (P) have recently increased, field experiments on this topic are lacking. Microcosm studies provided valuable insights, the lack of field studies represents a knowledge gap. Here, we present a field study in which we grew a mycorrhiza-defective tomato (*Solanum lycopersicum* L.) genotype (named *rmc*) and its mycorrhizal wild-type progenitor (named 76R) with and without additional fertilizer to examine the impacts of the AM symbiosis on soil P leaching and plant growth and nutrition. AM effects on fruit biomass and nutrients, soil nutrient availability, soil moisture and the soil bacterial community were examined. At the time of harvest, the AM tomato plants without fertilizer had the same fruit yield and fruit nutrients as plants that received fertilizer. The presence of roots reduced the concentration of available soil P, ammonium and soil moisture in the top 10 cm soil layer. Arbuscular mycorrhizas did not significantly affect soil nutrient availability, moisture, 16S bacterial community composition or leaching of P. These findings suggest an important role for AM fungi for tomato production but not necessarily for soil physicochemical traits, during the one season that this experiment was conducted. While longer-term field studies may be required in the future, the present study provides currently lacking insights into impacts of AM on P leaching in a field soil system, in a single growing season.

Key Words

Arbuscular mycorrhizal fungi (AMF); mycorrhiza-defective tomato mutant; phosphorus loss; tomato yield; soil moisture; soil bacterial community.

4.1 Introduction

Phosphorus (P) deficiency in agricultural soils is commonplace. The main causes of P deficiency in soils are P binding to soil particles and the formation of relatively insoluble precipitates with cations (White 2009). To overcome soil P deficiency, considerable amounts of P fertilizer are added to agricultural soils; however, typically only 10-30% of applied P is used by plants in the season of application (McLaughlin M J 1991; Cordell et al. 2009a). Phosphorus not taken up by plants is at risk of being lost from the system, for example via leaching, which can in turn lead to lead to eutrophication of water bodies (Kleinman et al. 2011; Sharpley et al. 2013).

Arbuscular mycorrhizal fungi (AMF) are widespread soil fungi that form associations with the roots of most (>80%) terrestrial plant species (Smith and Smith 2011). They can extend their external mycelium well beyond the root surface (Jakobsen et al. 1992), thereby exploring a larger soil volume than roots alone. The fungi are able to take up immobile nutrients, especially P, and deliver them to plants (Marschner and Dell 1994; Cavagnaro 2008). In return, the fungi receive a supply of carbon (C) from the plant (Berta et al. 2000; Nadejda et al. 2015). As arbuscular mycorrhizas (AM) can enhance plant P acquisition, they may also help reduce the risk of soil P loss via leaching (Parihar et al. 2019).

The potential for AM to reduce soil P leaching has been the subject of growing interest, with a number of studies having been undertaken to explore the impacts of AM on soil P loss via leaching (Cavagnaro et al. 2015). Such studies have included a variety of plant species and systems, including maize crops (Verbruggen et al. 2012), grazed grasslands (Kohl et al. 2014) and paddy rice (Zhang et al. 2020). A range of experimental approaches have also been employed, such as re-packed cores (Tran et al. 2020; Asghari and Cavagnaro 2012), nursery containers (Corkidi et al. 2011) and field lysimeters (Bender and van der Heijden 2015). While important insights have been gained, glasshouse-based studies have limitations. For example, the volume of soil in the pots might limit root growth and functioning (Bowles et al. 2016), re-packing of soil in pots or cores can change soil bulk density and structure, which can alter soil P leaching dynamics (Liu et al. 2012), and rainfalls simulator may not sufficiently reflect realistic rainfall events (Köhl and van der Heijden 2016). What is currently lacking are field-based studies that investigate the impact of AM on soil P leaching under realistic conditions (Bowles et al. 2017a; He et al. 2021).

Conducting field-based studies of AM on soil P leaching presents unique challenges. Non-mycorrhizal control treatments are typically established by sterilising soil, which eliminates other soil biota (Watts-Williams and Cavagnaro 2015). One way of overcoming this is the use of

mycorrhiza-defective mutants and their mycorrhizal wild-type progenitors (Rillig et al. 2008). The mycorrhiza-defective tomato (*Solanum lycopersicum* L.) mutant, named *rmc*, and its mycorrhizal wild type progenitor, named 76R, (Barker et al. 1998a) have been used as an effective tool for establishing non-mycorrhizal controls in the field (Bowles et al. 2016; Cavagnaro et al. 2006) and in the glasshouse (Bowles et al. 2017a; Cavagnaro et al. 2004). The use of these tomato genotypes makes it possible to investigate the influence of AM on nutrient leaching under realistic field conditions with the wider soil biota intact. This is important as soil microbes aside from AMF play an important role in soil P cycling (Paul 2014). While some studies have investigated such interactions (Rillig et al. 2006b; Marschner et al. 2001; Ames et al. 1984), none have done so in the context of AMF impacts on soil nutrient loss.

Soil nutrient leaching is strongly dependent on the movement of the soil solution though the profile (Djodjic et al. 2004). Arbuscular mycorrhizas can improve soil structure (Rillig and Mummey 2006), soil aggregation and water retention (Cavagnaro et al. 2006; Augé 2004), which in turn may decrease soil nutrient leaching (Cavagnaro et al. 2015). Soil moisture dynamics are important in influencing soil physicochemical properties, soil nutrient availability and the formation of AM and plant mycorrhizal responsiveness (Panhwar et al. 2019; Cavagnaro 2016). Importantly, soil moisture affects the concentration of available nutrients in soil solution (Cameron et al. 2013) and, thus, the amount of nutrient leached. While the effects of AM on the final leachate volume and nutrient concentrations in leachates are well documented (van der Heijden 2010; Asghari and Cavagnaro 2011; Köhl and van der Heijden 2016; Corkidi et al. 2011; Bender and van der Heijden 2015; Bender et al. 2014), changes in soil moisture down the soil profile during plant growth have not been studied in detail.

Here we present results of a field study where the mycorrhiza-defective tomato mutant genotype *rmc* and its mycorrhizal wild-type progenitor (76R) were grown with and without added mono-ammonium phosphate (MAP) fertilizer. An un-planted control was also included in the experiment. The main aim was to study the ecology of AM on soil P leaching under the field condition. We also looked at the impacts of AM on plant growth indicators, soil moisture and the diversity of soil bacterial community; and thus; how these changes related to mycorrhizal effects on soil P loss. Specifically, there were four research questions:

i. How do mycorrhizal roots and non-mycorrhizal roots affect soil P leaching loss under field condition?

ii. How do plant biomass, yield, and nutrient uptake of a mycorrhiza-defective tomato mutant plant and its mycorrhizal wild-type progenitor vary with phosphorus fertilizer application?

iii. How do mycorrhizal roots and non-mycorrhizal roots affect soil moisture and soil bacterial diversity?

iv. To what extent do changes in plant growth, soil moisture and soil (16S) bacterial community composition relate to soil P loss via leaching?

4.2 Materials and methods

4.2.1 Field site and experimental design

The experiment was conducted in a large outdoor enclosure enclosed with bird-proof netting at Waite Campus, the University of Adelaide, South Australia (-34.966165, 138.633325), from 5 December 2019 to 12 February 2020 (days of transplantation and harvest, respectively). The mean monthly rainfall from December to February in 2020 was 29.2 mm, mean temperature was 23.3 ^{o}C (maximum) and 13.3 ^{o}C (minimum), with a maximum of 45.2 ^{o}C and a minimum of 9.4 ^{o}C (Australian Government, Bureau of Meteorology, http://www.bom.gov.au, last accessed May 2021). The rainfall and temperature data was collected from the Beaumont weather station (approx. 5.5 km from the field site) and the Kent Town station (approx. 6 km from the field site), respectively.

The soil was fine sandy loam, Urrbrae red-brown earth (Alfisol) with a total P concentration of 693 ± 15 mg kg^{-1} dry soil, plant-available (Colwell) P concentration of 158 ± 4.5 mg kg^{-1} dry soil, nitrate concentration of 19 ± 3.1 mg kg^{-1} dry soil and ammonium concentration of 1.5 ± 0.1 mg kg^{-1} dry soil (see below for analytical methods). The soil was rewetted and ploughed to eliminate residual weeds prior to transplanting the tomato seedlings.

Non-mycorrhizal control and mycorrhizal plant treatments were established using a mycorrhiza-defective tomato (*Solanum lycopersicum* L.) mutant with reduced mycorrhizal colonization (*rmc* hereafter), and its mycorrhizal wild-type progenitor (76R hereafter) (Barker et al. 1998a). Seeds of the 76R and *rmc* tomato genotypes were shaken in a 10% sodium hypochlorite solution to surface-sterilise the seeds and then rinsed with reverse osmosis (RO) water. The seeds were sown in into a coarse/fine sand mixture (3:1 w/w) for germination. After one week, when green cotyledons had emerged, the seedlings were transferred to one litre pots

containing BioGro compost (Van Schaik's BioGro, 270 Wandilo Forest Rd, Wandilo SA) and grown in a controlled environment glasshouse for four weeks. The plants were moved to a net house next to the field site one week before transplanting to the field site.

The experiment was implemented as a randomised complete block design in a 12 m x 6 m section of the netted outdoor enclosure. The experiment was set up with two soil fertilizer levels (see below for fertilizer information), the two tomato genotypes (*rmc* and 76R) and a no-plant control, giving six different treatments, with a total of 36 plots across six blocks. Each block was separated by a one-meter buffer and included six beds (0.5 m wide x 0.8 m length). Each plot was one meter apart and contained three plants. The middle plant was instrumented (with the anion-exchange resin membranes and the soil moisture access tubes, see below) and assigned as the experimental plants. The other two plants were two buffer plants. The seedlings were transplanted in rows (40 cm apart). In total, there were 36 experimental plants and 72 buffer plants.

The resin-P method of Kouno et al. (1995) was used to estimate the soil P lost through leaching. Anion-exchange resin membrane (#0551642S, VWR International Ltd, England) was cut into 6 x 2 cm strips. The strips were prepared by shaking in 0.5 M hydrochloric acid for one hour, followed by two one-hour intervals in 0.5 M sodium bicarbonate ($NaHCO_3$) and washing with RO water. The strips then were shaken for one hour in freshly prepared 0.5M sodium bicarbonate ($NaHCO_3$) and washed with RO water. The prepared strips were stored in RO water until use. As most of the tomato root biomass is concentrated in the top 40 cm of the soil profile (Machado and Oliveira 2005), the anion-exchange resin membranes were placed at 30 cm soil depth (below the experimental plants) to estimate the P leached to this soil layer. A soil core (30 cm deep; 5 cm diameter) was taken at each site of planting and a resin strip was placed to the soil hole. The resin was connected by string to the soil surface to facilitate retrieval at harvest. Soil from the core was used to back-fill the holes.

Soil moisture was measured using a multi-profile soil moisture logger (Odyssey® Xtreem), inserted via 1 m long PVC access tubes installed so as to leave 8 cm of the tube above the soil level. These tubes were capped between soil moisture measurements. In total, 36 access tubes were installed 20 cm from each experimental plant. Soil moisture data was collected weekly from 15 days after transplanting.

Mono-ammonium phosphate (MAP) (22% P and 13% N) was used as the fertilizer source; levels of applied P and N were 30 kg ha^{-1} and 17.6 kg ha^{-1}, respectively. In the fertilizer plots, MAP was banded at 10 cm soil depth, below the plants. This approach was used to reduce

fertilizer surface run-off losses (Djodjic et al. 2005). An approx. 2 cm layer of soil was placed on the fertilizer band to avoid direct contact with roots of the transplanted seedlings. Immediately after applying the fertilizer, seedlings were transplanted into the beds.

A drip irrigation network consisting of a water pressure reducing valve, a timer (Node, Hunter Industries Incorporated) and "one-litre per hour" drippers was installed. Calibration indicated each dripper provided 50 mL water per minute at the water pressure used. Irrigation was scheduled three times a day using the timer; total water applied was approximately 400 mL $plant^{-1}$ day^{-1} following the recommendation of Harmanto et al. (2005) as crop water requirement for tomato under drip irrigation.

4.2.2 Plant and soil sampling

Aboveground plant biomass was harvested by cutting the stem at the soil level at 65 days after transplanting. Fruits (both green and red fruits) and shoots were separated and the fruit fresh weight (FFW) was determined. Shoot material was dried at 60 ^{o}C and shoot dry weight (SDW) determined. Following dry weight determination, plant material was ground to fine powder. The concentration of elements in shoots and fruits (Ca, K, Mg, S, Fe, Zn, Cu, Mn, Na, P) were analysed using an Avio 200 ICP Optical Emission Spectrometer (Perkin Elmer), following digestion with nitric acid and hydrogen peroxide (Wheal et al. 2011). The shoot and fruit samples were also analysed for nitrogen concentration (Dumas method; APAL-Australian Precision Ag Laboratory).

On the day of harvest, a soil core (30 cm deep; 5 cm diameter) was collected 5 cm from the stem of each experimental plant. The soil was separated into two layers: 0-10 cm and 10-30 cm. An approximately 25 g soil sample from each layer was taken for determination of total P, plant-available (Cowell P), soil ammonium and nitrate and soil gravimetric water content as follows. The concentration of total P in soil samples was analysed using an Avio 200 ICP Optical Emission Spectrometer (Perkin Elmer), following digestion with nitric acid and hydrochloric acid (Wheal et al. 2011). The concentration of plant-available (Colwell) P in soil samples was determined using a modification of Colwell (1963) (extraction with 0.5 M $NaHCO_3$ at a soil:extractant ratio of 1:100 and 16 hours shaking) followed by colorimetric analysis (Murphy and Riley 1962). The soil samples were analysed for ammonium and nitrate on 2 M potassium chloride extracts (APAL- Australian Precision Ag Laboratory).

Roots were retrieved from soil cores by wet sieving using RO water. Representative samples of fresh roots (approx. 0.5 g) were fixed in ethanol and then cleared with a 10 %

potassium hydroxide (*w/v*) solution at room temperature for seven days. Cleared roots were stained with 5 % ink in vinegar solution at 60 ^{o}C for 10 minutes (Vierheilig et al. 1998) before being de-stained in acidified water for 24 hours. Arbuscular mycorrhizal colonization of the roots was then determined using the gridline intersect method for at least 100 intersections (Giovannetti and Mosse 1980).

4.2.3 Phosphorus leaching

The buried resin strips were retrieved from the soil a day after the harvest day and stored in RO water in about 5 hours. The resin strips were rinsed with RO water to remove adhering soil, placed in 50 mL tubes and shaken horizontally for 2 hours in 30 mL of 0.1 M NaCl/HCl to elute P from the resin membrane (Butterly et al. 2011). The resin-extractable P concentration in the elution solution was measured by colorimetric analysis (Murphy and Riley 1962).

4.2.4 DNA extraction, amplification and sequencing

Genomic DNA (Illumina) sequencing was used to characterise the soil 16S bacterial community. The aim was to determine differences in soil bacterial communities between the mycorrhizal and non-mycorrhizal rhizosphere, and whether any changes could be linked to soil P availability, plant P uptake or P leached. Therefore, the 24 soil samples from plant treatments at 0-10 cm soil depth were used in the analysis.

Approximately 20 g soil was subsampled from the soil core at 0-10 cm and frozen at -20 ^{o}C prior to DNA extraction and lllumina sequencing. DNA extraction, PCR amplification and sequencing were conducted by the Australian Genome Research Facility (AGRF, Adelaide, Australia) as described in (Smith et al. 2018). Briefly, PowerSoil DNA Isolation Kits (MoBio Laboratories, Solana Beach, CA, USA) were used to extract DNA. Bacterial ribosomal RNA was analysed using forward and reverse primers 341F-806R (V3-V4)-CCTAYGGGRBGCASCAG and GGACTACNNGGGTATCTAAT, respectively. The PCR amplification conditions were as follows: an initial denaturation at 95^{o}C for 7 minutes and then 94^{o}C for 30 seconds, 55^{o}C for 45 seconds and 72^{o}C for 60 seconds repeated for 35 cycles with a final extension of 72^{o}C for 7 minutes.

Image analysis was performed using the MiSeq Control Software (MCS) v3.1.0.13 and Real Time Analysis (RTA) v1.18.54.4. Then the Illumina bcl2fastq 2.20.0.422 pipeline was used to generate the sequence data (followed AGRF's services report). The data generated met the AGRF quality standards and was formatted as FastQ sequence files containing a 300bp paired end run.

4.2.5 Bioinformatic analysis

Diversity profiling analysis was performed using Bioconductor packages (version 3.11) of R statistical software, version 4.0.2 (R Core Team, 2019). The bioinformatic analysis involved demultiplexing, quality control, OTU clustering, and taxonomic classification. The demultiplexed raw reads were primer trimmed and quality filtered followed by denoising with DADA2 pipeline (version 1.16) (Callahan et al. 2016). The sequence read lengths were trimmed (truncated) to 250 bp and 220 bp for forward read and reverse read, respectively. Trimming also occurred on 17 bp and 20 bp of left and right end reads, to remove low quality tails. Taxonomy was assigned to the amplicon sequences variant (ASVs) using decipher software (Wright 2016) and the IDTAXA (Murali et al. 2018) taxonomy classifier.

Taxonomic diversity is presented at family level with 12 classifications with equal to approximately 85 % of total abundance. Principle coordinate analysis (PCoA) plots (Bray-Curtis dissimilarity) were performed using the *plot_ordination* function of the phyloseq package (R statistical software, version 4.0.2). Alpha diversity expressed as Shannon and Simpson diversity, were estimated and plotted using *estimate_richness* and *plot_richness* functions in the phyloseq R package.

4.2.6 Statistical analysis

All statistical analysis was performed using R statistical software, version 4.0.2 (R Core Team, 2019). Soil, plant and gravimetric soil moisture data were checked for the assumption of normality by analysing model residuals using a QQ plot and Shapiro-Wilk test. A log transformation was used if the data did not comply with normal distribution. Two-way analysis of variance (ANOVA) was performed with *Fertilizer* treatment and *Plant* treatment (i.e. mycorrhizal plant, non-mycorrhizal plant, or no-plant), as factors in the analysis. In case of a significant interaction, means were compared using Tukey's HSD tests (at $\alpha < 0.05$). Differences in abundance within the most abundant family and alpha diversity treatments were also analysed using two-way ANOVA.

Targeted *Student's t*-tests were conducted in plant genotypes to identity mycorrhizal effects in each *Fertilizer* treatment. Any significant differences between the mycorrhizal and non-mycorrhizal means are denoted on the respective figure.

Soil moisture data measured from the multi-profile moisture logger was visualized using the *ggplot* function of the *tidyverse* package in R software.

4.3 Results

4.3.1 Mycorrhizal colonization, plant growth and nutrient uptake

While no arbuscular mycorrhizal colonization was recorded in the roots of the mycorrhiza-defective tomato genotype (*rmc*), the 76R roots were colonised by AMF. MAP addition had an antagonistic effect on arbuscular mycorrhizal colonization of the 76R plant roots (20 ± 3.6 % and 7.5 ± 2.5 % with -MAP and +MAP, respectively) (Figure 3).

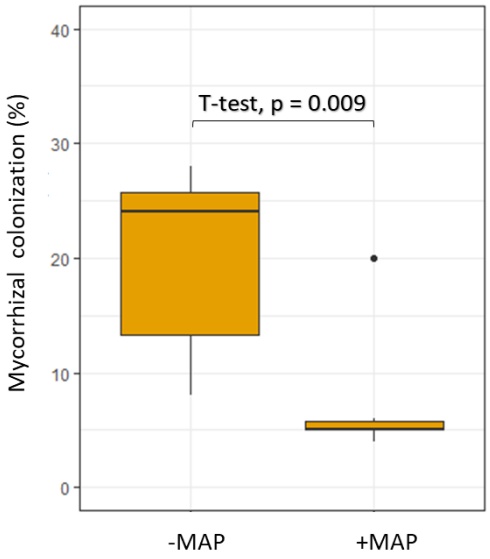

Figure 3. Mycorrhizal colonization of mycorrhizal plants (%). Horizontal lines indicate the median, box the interquartile range (IQR), whiskers extent to upper adjacent value (largest value=75th percentile +1.5 x IQR) and lower adjacent value (smallest value=25thpercentile -1.5xIQR), and dots represent outliers.

MAP application and plant genotype had no significant impact on shoot dry weight or fresh fruit weight. While MAP addition had no impact on P plant uptake in shoot and fruit, it significantly increased N shoot concentration (Figure 4).

The unfertilised (-MAP) 76R plants had fresh fruit yield, shoot dry weight, N shoot concentration and all fruit mineral nutrients (concentration and content) (Table 2 and 3) similar to that of plants that received MAP.

When targeted *t*-tests were performed to examine the effects of plant genotypes on fresh fruit weight for unfertilised plants, fresh fruit weight was significantly greater in the 76R genotype than *rmc* (Figure 4D).

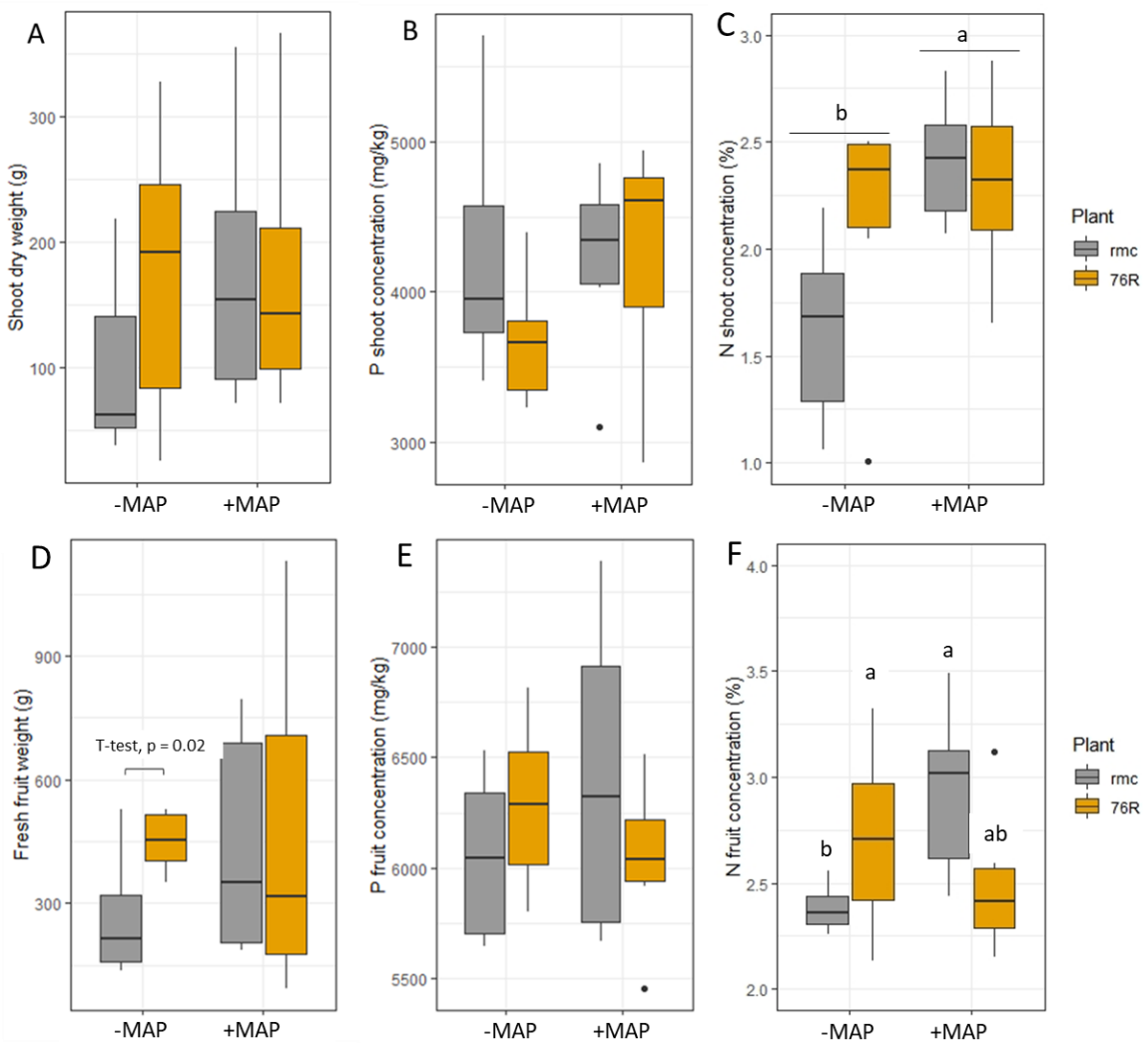

Figure 4. Shoot dry weight (g) (A), P shoot concentration (mg/kg) (B), N shoot concentration (%) (C), fresh fruit weight (g) (D), P fruit concentration (g plant^{-1}) (E) and N fruit concentration (%) (F) of mycorrhizal plants (76R) and mycorrhiza-defective tomato (*rmc*) genotypes, following the application of P treatment. For explanation of box plot refer to Figure 1. The different letters on top of each box plot indicate significant differences ($P < 0.05$).

4.3.2 Plant-available (Colwell) soil P, soil N mineral and resin-extractable P

There was a difference in plant-available (Colwell) soil P concentration at two soil depths (0-10 cm) and (10-30 cm). In the 0-10 cm soil layer, MAP fertilizer application resulted in greater available soil P concentration in the no-plant control treatments; however, there was no significant difference between mycorrhizal and non-mycorrhizal plants in terms of available soil P. The presence of plants reduced the available soil P for fertilised (+ MAP) plants. In the 10-30 cm soil layer, MAP application also increased available soil P, but the presence of plants did not decrease available soil P at this depth. The ammonium concentration in soil had a similar trend to the available soil P at both soil depths. The MAP fertilizer application increased the soil nitrate concentration in both soil layers (Figure 5).

There was no significant difference in resin-extractable P (at 30 cm) among the treatments. The resin-extractable P values were more variable for the fertilised than the unfertilised treatments. The amount of resin-extractable P in fertilised treatments was only slightly higher, but this difference was not significant (P = 0.15) (Figure 6).

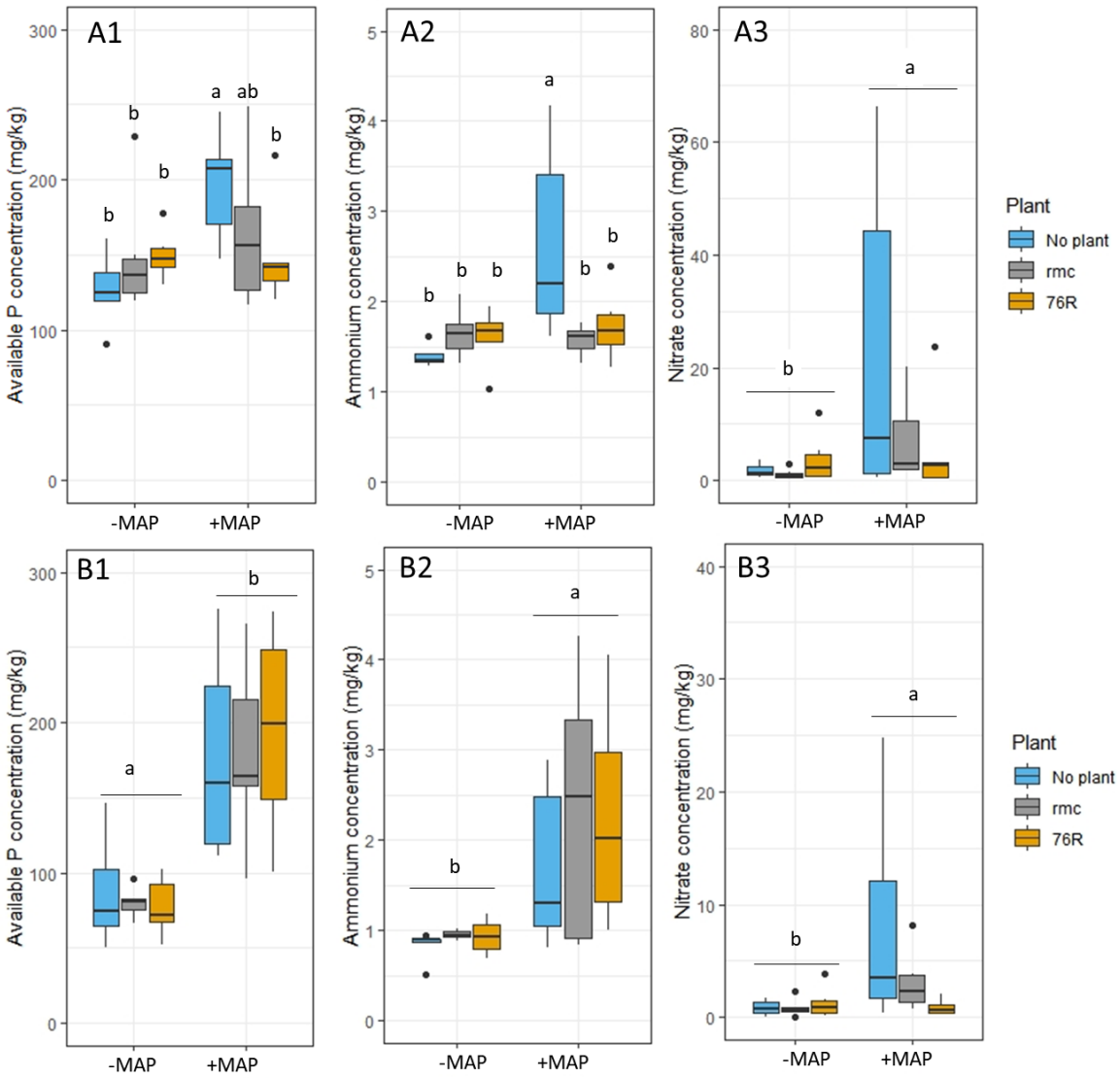

Figure 5. Plant available phosphorus, ammonium, nitrate concentration (mg kg^{-1}) in soil at 0-10 cm (A1, A2, A3) and 10-30 cm (B1, B2, B3) depth, respectively. The different letters on top of each box plot indicate significant differences ($P < 0.05$).

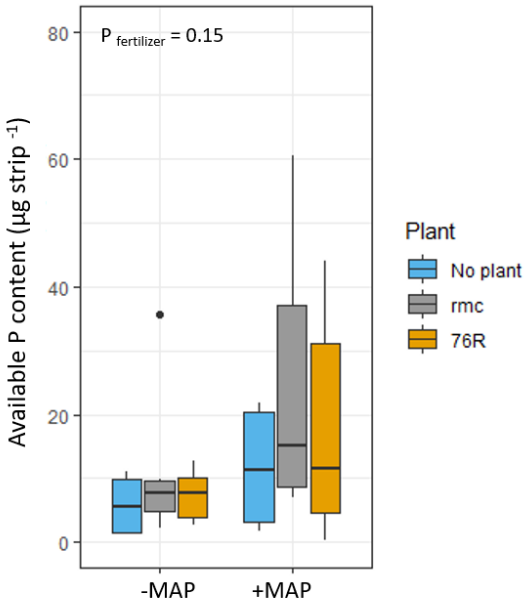

Figure 6. Available extractable P content (µg strip^{-1}) from the anion-exchange resin membranes

4.3.3 Soil moisture

a. Gravimetric soil moisture (0-30 cm)

In general, the gravimetric moisture of soils where MAP fertilizer was added were significantly lower than where no MAP fertilizer was added. Where MAP was added, plant-free treatments had a higher soil moisture than plant treatments in both 0-10 cm and 10-30 cm soil layers on harvest day. Where MAP was not added, there was a similar patterns of soil moisture but this pattern was less strong. There was no difference in gravimetric soil moisture between mycorrhizal and non-mycorrhizal plant treatments at either soil depth (Figure 7).

b. Soil moisture patterns (soil moisture probe, 0-100cm)

In general, soil moisture displayed the same pattern with depth among the treatments and was consistent throughout the experiment (Figure 8). Mean moisture content of soil across the whole experiment at 20 cm was lowest at 8.04 ± 0.18 %, followed by 12.20 ± 0.2 % at 40 cm and 15.36 % ± 0.14; 16.41 % ± 0.13 at 60 cm and 80 cm soil depth, respectively. There were no significant differences among treatments at the individual depths (See Supplementary Figure S2 for box plots of individual time points and treatments). There was more variation in soil moisture at the 20 cm and 40 cm soil depths than at depths of 60 cm, 80 cm and 100 cm.

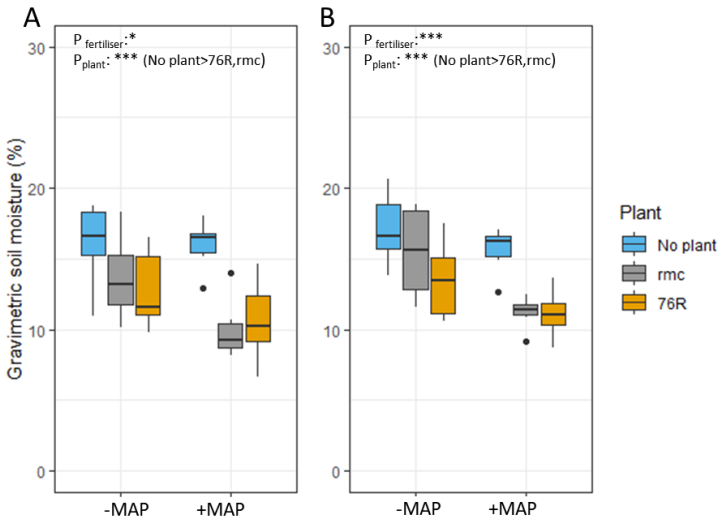

Figure 7. Gravimetric soil moisture (%) of soil samples collected on the harvest day at 0-10 cm (A) and 10-30 cm (B) soil depth.

4.3.4 Bacterial (16S) community

Sequencing of 16S amplicons from 24 soil samples yielded 1,423,297 paired-end Illumina MiSeq reads. Taxonomy was assigned to a total of 3271 ASVs; bacteria accounted for 81.7% of ASVs, 17.1 % were unassigned and Archaea accounted for 1.2 %. The 20 most abundant bacterial ASVs were dominated by 12 families, which represented 85% of total bacterial taxa. There was no difference in total baterial abundance composition among the treatments (See Figure 9 for bar plots of total bacterial abundance and 12 dominant families).

Bacterial community compostion was dominated by *Bryobacteraceae, Micrococcaceae, Sphingomonadaceae, Xanthobacteraceae* and *Nitrososphaeraceae* in all soil samples. These families accounted for about 60 % of bacterial composition; *Bryobacteraceae* and *Micrococcaceae* dominated, comprising 22.4 ± 2.0 % and 15.4 ± 3.5 % of total baterial community compostion, respectively (Figure 9).

No differences in abundance were detected among experimental treatments for the 12 most abundant families using two way ANOVA (with plant genotype and MAP addition as factors in the analysis). There was also no significant difference in 16S alpha diversity among treatments, measured as Shannon, Simpson and Chao 1 diversity (Figure 10A). When analysed with Bray-Curtis distance matrices, there was no clear separation among treatment groups (Figure 10B).

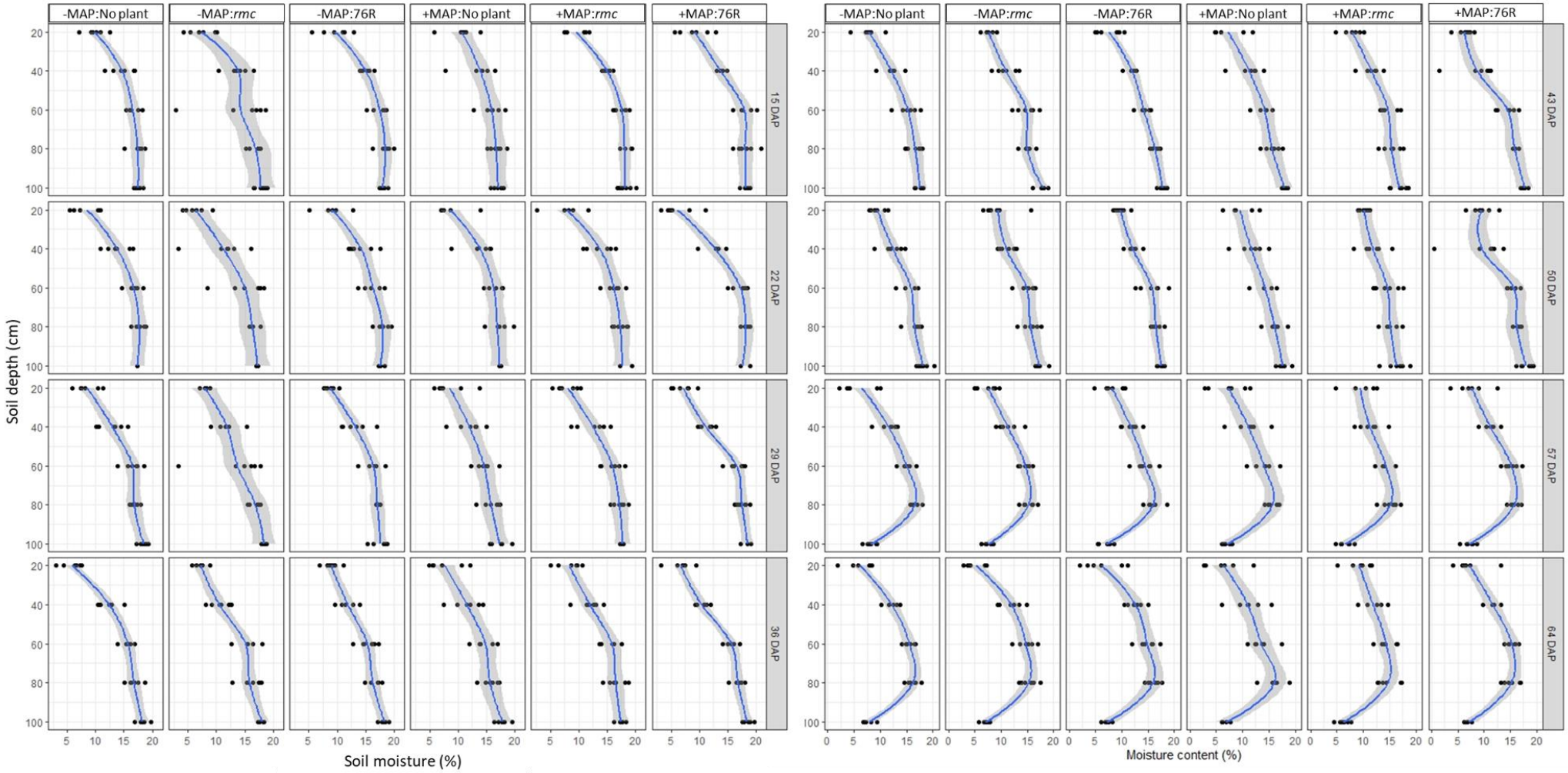

Figure 8. Soil moisture measure by the multi-profile soil moisture logger over the time (%). DAP is prefer to “day after transplanting”

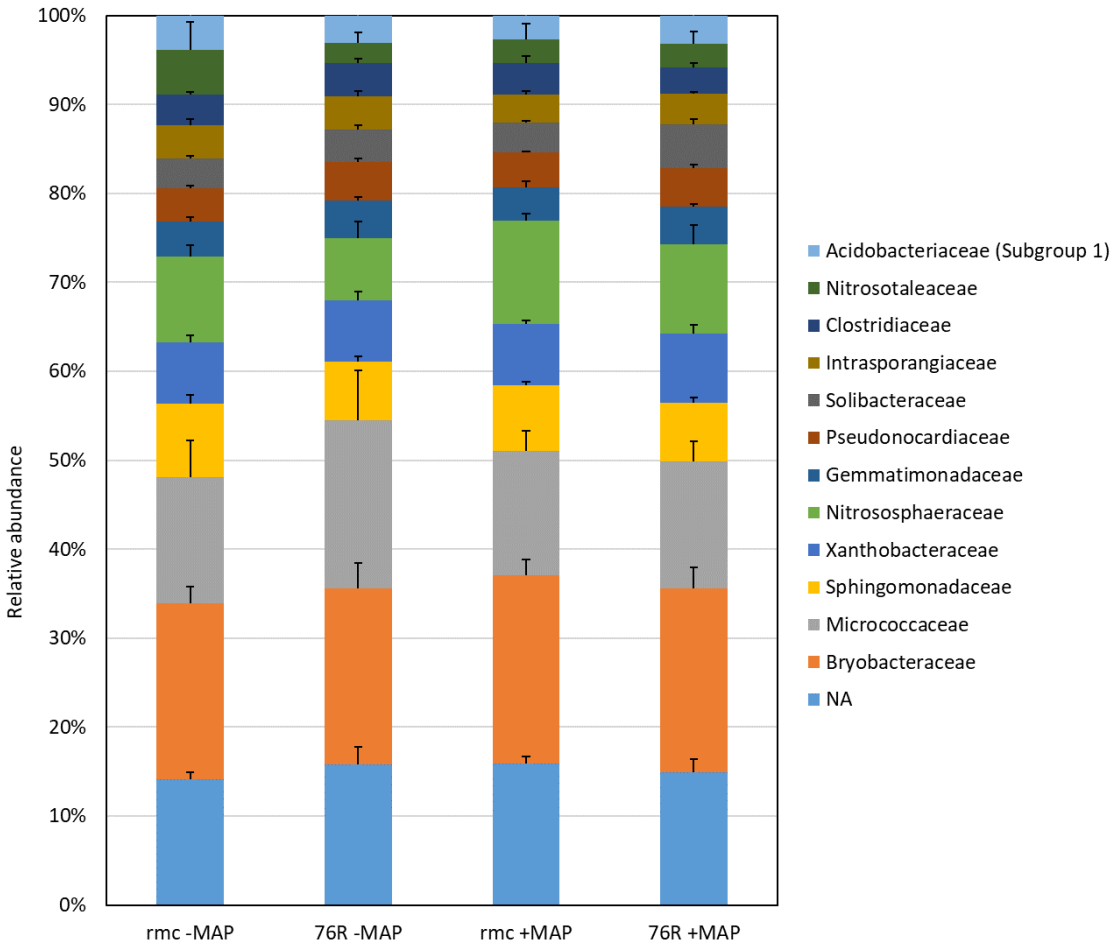

Figure 9. Bacterial community structure presented as composition and rare OTUs are displayed as “other" (N=24).

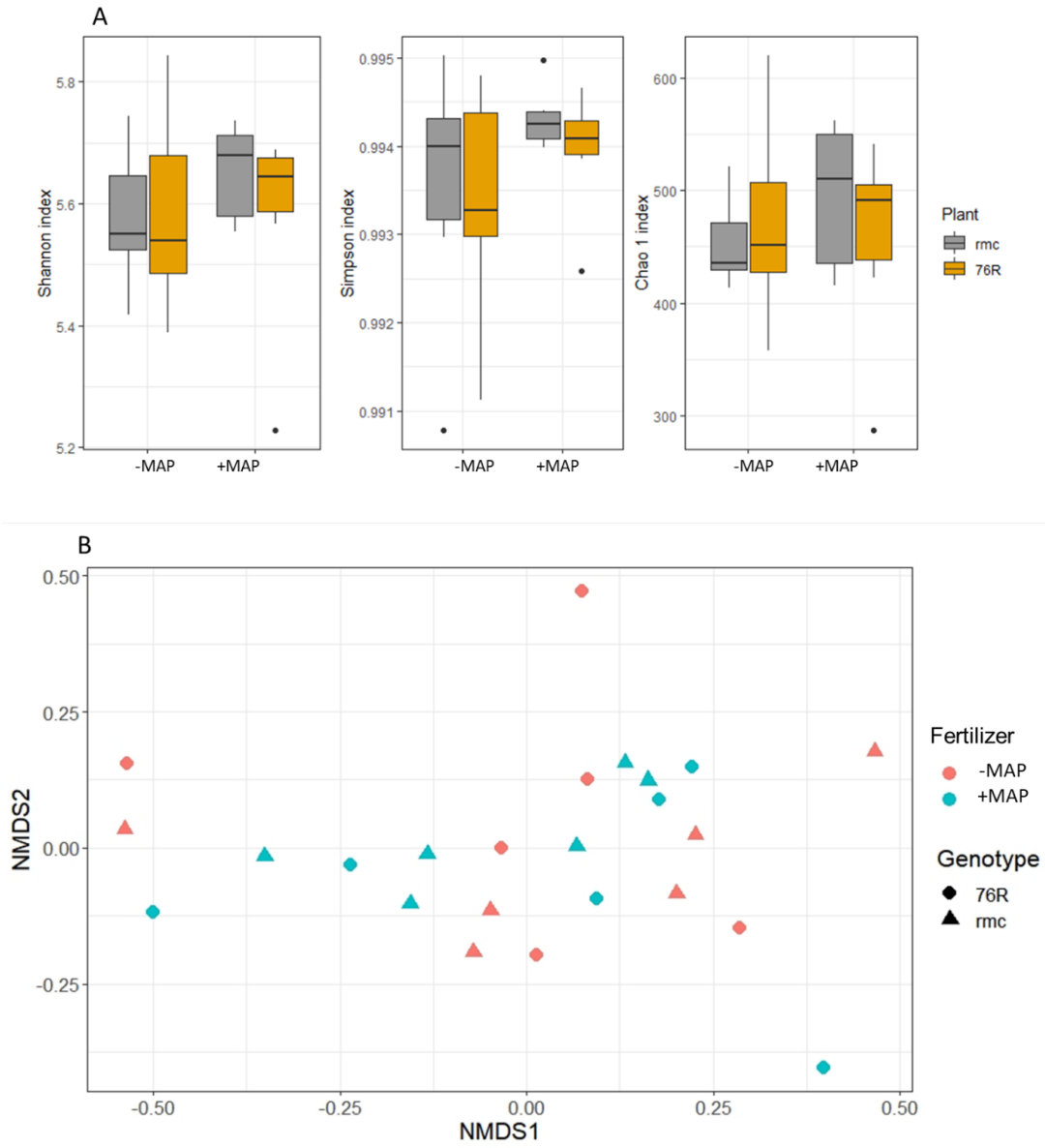

Figure 10. 16S alpha diversity (Shannon, Simpson and Chao 1 index) (A) and nonmetric multidimensional scaling (NMDS) (B) Bray-Curtis ordination of soil bacterial diversity (n=24).

Table 2. Nutrient contents of shoot and fruit tissues of *rmc* and 76R genotypes with and without MAP at harvest. Values are means ± SE, n=6

Nutrient content (g/plant)	-MAP		+MAP	
	rmc	76R	*rmc*	76R
Shoot				
P	0.44 (0.15)	0.64 (0.18)	0.74 (0.2)	0.70 (0.14)
N	1.82 (0.68)	4.13 (1.22)	4.40 (1.21)	4.24(1.37)
Fruits				
P	0.13 (0.03)	0.23 (0.02)	0.23 (0.06)	0.22 (0.07)
N	0.79 (0.19)	0.62 (0.10)	0.70 (0.22)	1.33 (0.32)
K	0.77 (0.20)	1.46 (0.15)	1.50 (0.41)	1.50 (0.58)
Ca	0.03 (0.01)	0.06 (0.01)	0.06 (0.02)	0.07 (0.03)
Mg	0.03 (0.01)	0.06 (0.01)	0.07 (0.02)	0.07 (0.03)
S	0.05 (0.01)	0.09 (0.01)	0.09 (0.03)	0.09 (0.04)
Fe	0.0007 (0.0002)	0.0014 (0.0002)	0.0017 (0.0004)	0.0014 (0.0006)
Zn	0.0006 (0.0004)	0.001 (0.0001)	0.001 (0.0003)	0.001 (0.0001)
Cu	0.0003(6.3E-05))	0.0005 (4.3E-05)	0.0005 (1.3E-04)	0.0005 (1.9E-04)
Mn	0.0003(7.34E-05)	0.0005 (4.38E-05)	0.0005 (0.0001)	0.0005 (0.0002)
Na	0.03 (0.008)	0.06 (0.003)	0.06 (0.015)	0.06 (0.023)

Table 3. Mineral nutrients concentration (mg/kg) of tomato fruits. Values are mean ± SE, n=6

Nutrient	-MAP		+MAP	
	rmc	76R	*rmc*	76R
K	35919 (1595)	39980 (1166)	41625 (1088)	38120 (1582)
Ca	1290 (80)	1718 (184)	1635 (96)	1786 (133)
Mg	1518 (65)	1728 (97)	1803 (72)	1647 (89)
S	2258 (60)	2442 (96)	2606 (50)	2410 (100)
Fe	30 (5.3)	38 (4.3)	51 (2.9)	31 (12.2)
Zn	30 (0.69)	32 (0.69)	33 (1.4)	32 (1.59)
Cu	12 (0.58)	13 (0.47)	13 (0.5)	12 (0.62)
Mn	11 (0.52)	13 (1.04)	13 (0.5)	12 (0.66)
Na	1524 (26.6)	1561 (22.6)	1596 (29.7)	1562 (28.1)

4.4 Discussion

To our knowledge, this is the first study to investigate the impact of AM ecology on soil P leaching under realistic field soil condition. The downward movements of P in soil profile were captured by the anion-exchange resin membrane at 30 cm soil depth. Whereas there was no significant difference in term of soil P leaching estimating by the amount of resin-extractable P between the mycorrhizal roots and non-mycorrhizal root systems, mycorrhizal tomato plants without fertilizer application had the same fruit yield and fruit nutrients as plants receiving MAP fertilizer. This highlights the potential beneficial ecosystem service of AM on improving tomato fruit biomass and nutrients and suggests the importance of maintaining AM formation in roots systems.

4.4.1 Arbuscular mycorrhizas increased tomato fruit yield and nutrients to the same degree as plants received fertilizer

Whereas the fruit yield of mycorrhizal and non-mycorrhizal plants did not differ in soils with MAP fertilizer, the yield of mycorrhizal plants was higher than that of non-mycorrhizal plants, where no MAP was applied. Moreover the yield of the mycorrhizal plants where no MAP was applied was as high as that of plants where MAP was applied. Interestingly, the greater fruit biomass of the mycorrhizal tomato plants was not associated with a greater shoot dry weight, or greater shoot P or N concentration. This highlights the beneficial effects of AM on the harvest index of mycorrhizal tomato plants, in which the allocation of plant resources to fruits over vegetative mass was prioritised in the 76R mycorrhizal plants relative to *rmc* non-mycorrhizal plants. This is consistent with a meta-analysis of field studies in Pellegrino et al. (2015) states that AMF can improve harvest index by 25%. Importantly, the fruit mineral nutrient content of the mycorrhizal plants where MAP was not applied, was similar to that of both genotypes where MAP was applied. These findings are in agreement with earlier work showing an increase in the fruit nutrient of mycorrhizal tomato plants (Hart et al. 2014; Cavagnaro et al. 2006). In addition, a similar yield response was found in another field experiment using the same tomato genotypes when subjected to droughted and non-droughted conditions (Bowles et al. 2016). Together these studies suggest that employing management practices that maintain and enhance the formation of AM in crop roots may reduce the need for external fertilizer inputs, as well as help buffer against drought stress.

It is well established that increasing levels of plant-available P in soil negatively affects mycorrhizal colonization of roots (Wheeler et al. 1992; M.H et al. 1994; Gianinazzi et al. 1990). In the present study, MAP application decreased mycorrhizal colonization of the mycorrhizal

wild-type progenitor by more than 60% compared to the control where no MAP was applied; colonization of 76R roots in the un-fertilised control was 20 % of root length, which is in line with previous field experiments using the same genotype (Cavagnaro et al. 2006; Bowles et al. 2016). Importantly, the roots of the *rmc* genotype were not colonised, and so it is possible to make a valid comparison of AM effects on plant growth, yield and nutrition, soil P leaching, soil (16S) microbial communities, and soil moisture, as follows.

4.4.2 Arbuscular mycorrhizas had no significant impacts on soil moisture, soil 16S bacterial community and soil P leaching.

In our study, the presence of roots reduced the gravimetric soil moisture content (in both the 0-10 and 10-30cm soil layers), and this effect was greatest with MAP addition. Fertilizer application might improve plant water uptake capacity as this can enhance plant biomass and photosynthesis (Wiedenfeld and Enciso 2008; Drerup et al. 2019; Dong et al. 2011). However, we did not observe an increase in above-ground biomass with MAP addition, and so the difference may be associated with a greater root biomass, or greater rates of transpiration. This, however, is speculative and warrants further investigation. In addition to assessing gravimetric moisture content at the time of harvest, we also used capacitance probes to monitor root and mycorrhizal effects on soil moisture at 20 cm increments to a depth of 1 m on a fortnightly basis over the course of the experiment. Although previous work has shown that AMF can alter soil moisture due to the activity of the fungal mycelium to increase plant water uptake (Khalvati et al. 2005) or soil water repellence (Rillig et al. 2010), we found no such mycorrhizal effects on soil moisture. The clear lack of root, or mycorrhizal effects on soil moisture may be due to the plants in this study being well-irrigated, in contrast to earlier studies focused on deficit irrigation (Bowles et al. 2016) or drought stress conditions (Aliasgharzad et al. 2006).

Although previous studies have demonstrated that the formation of AM can alter soil microbial communities (Xu et al. 2018; Zhang et al. 2019; Gui et al. 2017), we found that neither mycorrhizal colonization of tomato roots, nor MAP addition, resulted in a change in 16S (bacterial and archaea) community composition; this conclusion is based on our measures of 'species' (OTU) richness, alpha-diversity (Shannon, Simpson and Chao 1 index), and the NMDS plots. The lack of difference in soil bacterial communities among treatments may be due to the short experimental time frame (one field season) (Huang et al. 2014). It is also important to note that DNA-based methods may also detect DNA from recently dead cells (Andreas and Anne 2006; Lemarchand et al. 2004), thereby potentially masking shifts in community composition. Our findings here are also consistent with an earlier study indicating that arbuscular

mycorrhizas, irrespective of N and P addition, had no impact on soil ammonia oxidizing bacteria groups while using the same two tomato genotypes plants (Cavagnaro et al. 2007). Furthermore, in contrast to other studies where soil microbiomes have been found to shift in composition with changes in the level of soil nutrient availability (Leff et al. 2015; Mello et al. 2016), no impacts of MAP addition on the soil bacterial community were seen here.

The ion exchange resin technique has been used as an effective tool for assessing nutrient movement in soils in previous studies (Qian and Schoenau 2002; Pampolino et al. 2000). In this experiment, based on the amount of extractable P from the anion-exchange resin membranes, there were also no clear mycorrhizal, nor root (i.e. no plant controls), effects on soil P leached at 30 cm soil layer. This might be explained by the limitation of root effects on soil nutrients at different soil layers and slow movement of P in this soil context. In our previous leaching studies, we found a significant contribution of roots to the amount and composition of P leached (Tran et al. 2020). However, the presence of plants reduced soil nutrient concentrations (plant-available P and ammonium) in the 0-10 cm soil layer, but not the 10-30 cm soil layer, where the resin membranes were located. It is likely that the greater root biomass in the upper soil layer resulted in greater uptake of nutrients from this layer. Given that roots show a plastic response to soil P supply (Grossman and Rice 2012; Ram et al. 2000) and thus root distribution (Xia et al. 2013), this is not unexpected. In addition, the MAP was banded at a depth of 10 cm, so any movement of nutrients was likely downward into the lower soil layer. Any such downward movement of P was, however, limited, as indicated by only slightly higher (non-significant) levels of P in the resin strops deployed at a depth of 30 cm in the plots where MAP was applied. Therefore, the contribution of roots to the amount of soil P throughout soil profile (i.e at 0-10 cm and 10-30 cm soil layer) and movement of P in soil should be taken into consideration in future research on the topic of roots and mycorrhizal effects on soil nutrient loss via leaching.

4.5 Conclusions

This study provides further field-based evidence of the benefits of forming AM in terms of tomato yield and nutrition. Colonization of tomato roots by AMF in soils not receiving MAP resulted in an equivalent fruit yield and nutrient concentrations to plants (mycorrhizal and non-mycorrhizal) receiving MAP. On the other hand, fertilizer addition suppressed mycorrhizal colonization and had little impact on plant growth and nutrient uptake. This suggests that management practices that support AM colonization, such as cover crops and reduced tillage (Bowles et al. 2017b; Ramos-Zapata et al. 2012; Higo et al. 2013), may help reduce the need for inputs of fertilizers.

To our knowledge, this is the first field-based study to investigate the impact of AM on soil P leaching in association soil P availability, soil moisture and the composition of the soil 16S bacterial community. However, due to the time limitation, this experiment was only conducted in a short-term period (a tomato crop period). Therefore, there is a need for long-term studies focusing on AM impacts on P loss under field condition. Moreover, the high-throughput sequencing methods to study fungal diversity have been currently developed (Taylor et al. 2016). Therefore, the AM effects on soil fungal community should also be included in the future when studying AM impacts on soil microbial communities.

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Supplementary data

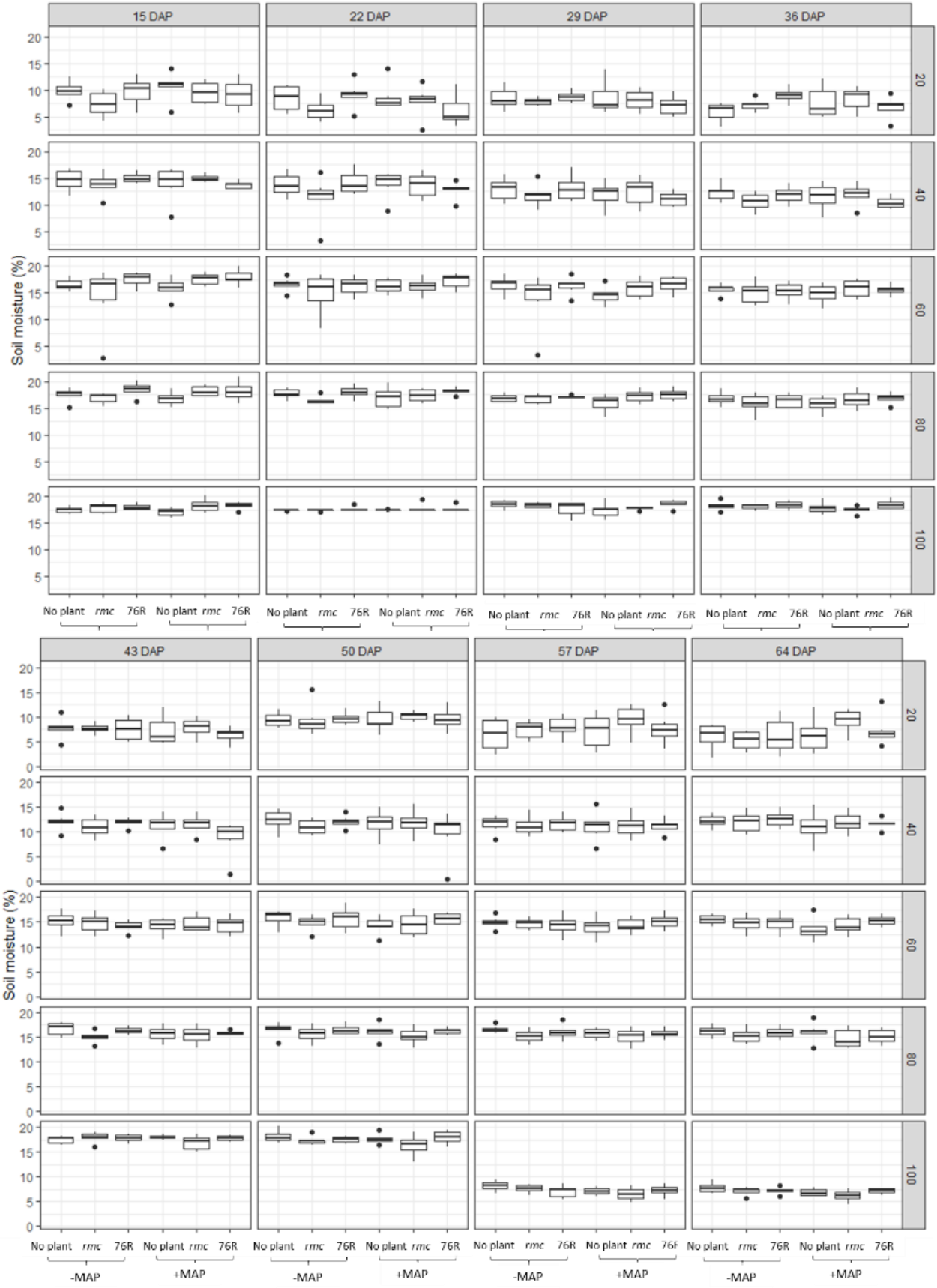

Figure S2. Boxplot of soil moisture at individual time points and treatments.

References

Aliasgharzad N, Neyshabouri MR, Salimi G (2006). Effects of arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* on drought stress of soybean. Biológia 61 (S19):S324-S328. doi:10.2478/s11756-006-0182-x

Ames RN, Reid CPP, Ingham ER (1984). Rhizosphere bacterial population responses to root colonization by a vesicular-arbuscular mycorrhizal fungus. The New phytologist 96 (4):555-563

Andreas N, Anne KC (2006). Selective removal of DNA from dead cells of mixed bacterial communities by use of ethidium monoazide. Applied and Environmental Microbiology 72 (3):1997-2004. doi:10.1128/AEM.72.3.1997-2004.2006

Asghari HR, Cavagnaro TR (2011). Arbuscular mycorrhizas enhance plant interception of leached nutrients. Functional Plant Biology 38 (3):219-226. doi:10.1071/FP10180

Asghari HR, Cavagnaro TR (2012). Arbuscular mycorrhizas reduce nitrogen loss via leaching Ploss one 7 (1)

Augé RM (2004). Arbuscular mycorrhizae and soil/plant water relations. Canadian journal of soil science 84 (4):373-381. doi:10.4141/S04-002

Barker SJ, Stummer B, Gao L, Dispain I, O'Connor PJ, Smith SE (1998). A mutant in Lycopersicon esculentum Mill. with highly reduced VA mycorrhizal colonization: isolation and preliminary characterisation. The Plant Journal 15 (6):791-797. doi:doi:10.1046/j.1365-313X.1998.00252.x

Bender SF, Conen F, Van der Heijden MGA (2014). Mycorrhizal effects on nutrient cycling, nutrient leaching and N2O production in experimental grassland. Soil Biology and Biochemistry 80 (Supplement C):283-292. doi:https://doi.org/10.1016/j.soilbio.2014.10.016

Bender SF, van der Heijden MGA (2015). Soil biota enhance agricultural sustainability by improving crop yield, nutrient uptake and reducing nitrogen leaching losses. Journal of Applied Ecology 52 (1):228-239. doi:10.1111/1365-2664.12351

Berta B, Philip EP, Yair S-H (2000). Carbon metabolism and transport in arbuscular mycorrhizas. Plant physiology (Bethesda) 124 (3):949-957. doi:10.1104/pp.124.3.949

Bowles TM, Barrios-Masias FH, Carlisle EA, Cavagnaro TR, Jackson LE (2016). Effects of arbuscular mycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. The Science of the total environment 566-567:1223-1234. doi:10.1016/j.scitotenv.2016.05.178

Bowles TM, Jackson LE, Cavagnaro TR (2017a). Mycorrhizal fungi enhance plant nutrient acquisition and module nitrogen loss with variable water regimes. Global Change Biology. doi:10.1111/gcb.13884

Bowles TM, Jackson LE, Loeher M, Cavagnaro TR, Nuñez M (2017b). Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. The Journal of applied ecology 54 (6):1785-1793. doi:10.1111/1365-2664.12815

Butterly C, McNeill A, Baldock J, Marschner P (2011). Rapid changes in carbon and phosphorus after rewetting of dry soil. Biology and Fertility of Soils 47 (1):41-50. doi:10.1007/s00374-010-0500-x

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature methods 13 (7):581-583. doi:10.1038/nmeth.3869

Cameron KC, Di HJ, Moir JL (2013). Nitrogen losses from the soil/plant system: a review. Annals of applied biology 162 (2):145-173. doi:10.1111/aab.12014

Cavagnaro TR (2008). The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: A review. Plant and Soil 304 (1-2):315-325. doi:10.1007/s11104-008-9559-7

Cavagnaro TR (2016). Soil moisture legacy effects: Impacts on soil nutrients, plants and mycorrhizal responsiveness. Soil biology & biochemistry 95:173-179. doi:10.1016/j.soilbio.2015.12.016

Cavagnaro TR, Bender SF, Asghari HR, van der Heijden MGA (2015). The role of arbuscular mycorrhizas in reducing soil nutrient loss. Trends in Plant Science 20 (5):283-290. doi:10.1016/j.tplants.2015.03.004

Cavagnaro TR, Jackson LE, Scow KM, Hristova KR (2007). Effects of Arbuscular Mycorrhizas on Ammonia Oxidizing Bacteria in an Organic Farm Soil. Microbial ecology 54 (4):618-626. doi:10.1007/s00248-007-9212-7

Cavagnaro TR, Jackson LE, Six J, Ferris H, Goyal S, Asami D, Scow KM (2006). Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. Plant and Soil 282 (1):209-225. doi:10.1007/s11104-005-5847-7

Cavagnaro TR, Smith FA, Hay G, Carne-Cavagnaro VL, Smith SE (2004). Inoculum type does not affect overall resistance of an arbuscular mycorrhiza-defective tomato mutant to colonisation but inoculation does change competitive interactions with wild-type tomato. New Phytologist 161 (2):485-494. doi:doi:10.1111/j.1469-8137.2004.00967.x

Colwell JD (1963). The estimation of phosphorus fertiliser requirements of wheat in southern New South Wales Australian. Journal of Experimental Agriculture:190-197

Cordell D, Drangert J-O, White S (2009). The story of phosphorus: global food security and food for thought. Global Environmental Change 19 (2):292-305. doi:10.1016/j.gloenvcha.2008.10.009

Corkidi L, Merhaut DJ, Allen EB, Downer J, Bohn J, Evans M (2011). Effects of mycorrhizal colonization on nitrogen and phosphorus leaching from nursery containers. HortScience 46 (11):1472-1479

Djodjic F, Bergström L, Grant C (2005). Phosphorus management in balanced agricultural systems. Soil Use and Management 21:94-101. doi:10.1111/j.1475-2743.2005.tb00413.x

Djodjic F, Börling K, Bergström L (2004). Phosphorus leaching in relation to soil type and soil phosphorus content. Journal of Environmental Quality 33 (2):678-684. doi:10.2134/jeq2004.6780

Dong W, Qin J, Li J, Zhao Y, Nie L, Zhang Z (2011). Interactions between soil water content and fertilizer on growth characteristics and biomass yield of Chinese white poplar (*Populus tomentosa* Carr.) seedlings. Soil science and plant nutrition (Tokyo) 57 (2):303-312. doi:10.1080/00380768.2010.549445

Drerup P, Brueck H, Eticha D, Scherer HW (2019). NDVI-based estimates of evapotranspiration of winter wheat indicate positive effects of nitrogen fertilizer application on agronomic water-use efficiency. Journal of agronomy and crop science (1986) 206 (1):1-12. doi:10.1111/jac.12364

Gianinazzi S, Trouvelot A, Gianinazzi-Pearson V (1990). Conceptual approaches for the rational use of VA endomycorrhizae in agriculture: possibilities and limitations. Agriculture, ecosystems and environment 29 (1):153-161. doi:10.1016/0167-8809(90)90269-J

Giovannetti M, Mosse B (1980). Evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist 84:498-500

Grossman JD, Rice KJ (2012). Evolution of root plasticity responses to variation in soil nutrient distribution and concentration. Evolutionary applications 5 (8):850-857. doi:10.1111/j.1752-4571.2012.00263.x

Gui H, Hyde K, Xu J, Mortimer P (2017). Arbuscular mycorrhiza enhance the rate of litter decomposition while inhibiting soil microbial community development. Scientific reports 7 (1):42184-42184. doi:10.1038/srep42184

Harmanto, Salokhe VM, Babel MS, Tantau HJ (2005). Water requirement of drip irrigated tomatoes grown in greenhouse in tropical environment. Agricultural Water Management 71 (3):225-242. doi:10.1016/j.agwat.2004.09.003

Hart M, Ehret DL, Krumbein A, Leung C, Murch S, Turi C, Franken P (2014). Inoculation with arbuscular mycorrhizal fungi improves the nutritional value of tomatoes. Mycorrhiza 25 (5):359-376. doi:10.1007/s00572-014-0617-0

He Y, Yang R, Lei G, Li M, Li T, Zhan F, Li Y (2021). Arbuscular mycorrhizal fungus--induced decrease in phosphorus loss due to leaching in red soils under simulated heavy rainfall. Journal of soils and sediments 21 (2):881. doi:10.1007/s11368-020-02849-z

Higo M, Isobe K, Yamaguchi M, Drijber RA, Jeske ES, Ishii R (2013). Diversity and vertical distribution of indigenous arbuscular mycorrhizal fungi under two soybean rotational systems. Biology and fertility of soils 49 (8):1085-1096. doi:10.1007/s00374-013-0807-5

Huang YZ, Zhong M, Wu W, Sui LH, Zhang C, Hao XW (2014). Effects of arbuscular mycorrhizal fungi isolated from white clovers (*Trifolium repens* L) on soil bacteria and fungi. Chemistry in ecology 30 (2):118-132. doi:10.1080/02757540.2013.841892

Jakobsen I, Abbott LK, Robson AD (1992). External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. The New Phytologist 120 (3):371-379. doi:10.1111/j.1469-8137.1992.tb01077.x

Khalvati MA, Hu Y, Mozafar A, Schmidhalter U (2005). Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations, and

gas exchange of barley subjected to drought stress. Plant biology (Stuttgart, Germany) 7 (6):706-712. doi:10.1055/s-2005-872893

Kleinman PJA, Sharpley AN, Buda AR, McDowell RW, Allen AL (2011). Soil controls of phosphorus in runoff: management barriers and opportunities. Canadian journal of soil science 91 (3):329-338. doi:10.4141/cjss09106

Köhl L, van der Heijden MGA (2016). Arbuscular mycorrhizal fungal species differ in their effect on nutrient leaching. Soil Biology and Biochemistry 94:191-199. doi:10.1016/j.soilbio.2015.11.019

Kohl L, van der Heijden MGA, Oehl F (2014). Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota.(Report). Ecological Applications 24 (7)

Kouno K, Tuchiya Y, Ando T (1995). Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. Soil Biology and Biochemistry 27 (10):1353-1357. doi:10.1016/0038-0717(95)00057-L

Leff JW, Jones SE, Prober SM, Barberán A, Borer ET, Firn JL, Harpole WS, Hobbie SE, Hofmockel KS, Knops JMH, McCulley RL, La Pierre K, Risch AC, Seabloom EW, Schütz M, Steenbock C, Stevens CJ, Fierer N (2015). Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proceedings of the National Academy of Sciences - PNAS 112 (35):10967-10972. doi:10.1073/pnas.1508382112

Lemarchand K, Masson L, Brousseau R (2004). Molecular biology and DNA microarray technology for microbial quality monitoring of water. Critical reviews in microbiology 30 (3):145-172. doi:10.1080/10408410490435142

Liu J, Aronsson H, Bergström L, Sharpley A (2012). Phosphorus leaching from loamy sand and clay loam topsoils after application of pig slurry. SpringerPlus 1 (1):1-10. doi:10.1186/2193-1801-1-53

M.H R, G.A C, D.C D (1994). Colonisation of wheat by VA-mycorrhizal fungi was found to be higher on a farm managed in an organic manner than on a conventional neighbour. Plant and soil 160 (1):33-40. doi:10.1007/BF00150343

Machado RMA, Oliveira MdRG (2005). Tomato root distribution, yield and fruit quality under different subsurface drip irrigation regimes and depths. Irrigation science 24 (1):15-24. doi:10.1007/s00271-005-0002-z

Marschner H, Dell B (1994). Nutrient uptake in mycorrhizal symbiosis. Plant and Soil 159 (1):89-102. doi:10.1007/BF00000098

Marschner P, Crowley D, Lieberei R (2001). Arbuscular mycorrhizal infection changes the bacterial 16 S rDNA community composition in the rhizosphere of maize. Mycorrhiza 11 (6):297-302. doi:10.1007/s00572-001-0136-7

McLaughlin MJ, Fillery IR, Till AR, (1992). Operation of the phosphorus , sulphur and nitrogen cycles In: RM Gifford and M.M. Barson (ed) Australia's renewable resources : sustainability and global change. Bureau of rural resources, Canbera, Autralia, pp 67-116

Mello BL, Alessi AM, McQueen-Mason S, Bruce NC, Polikarpov I (2016). Nutrient availability shapes the microbial community structure in sugarcane bagasse compost-derived consortia. Scientific reports 6 (1):38781-38781. doi:10.1038/srep38781

Murali A, Bhargava A, Wright ES (2018). IDTAXA: a novel approach for accurate taxonomic classification of microbiome sequences. Microbiome 6 (1):140-114. doi:10.1186/s40168-018-0521-5

Murphy J, Riley JP (1962). A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27:31-36. doi:https://doi.org/10.1016/S0003-2670(00)88444-5

Nadejda AS, Marcel GAvdH, Johannes HCC, Mikhail IM, Vladimir GO, Mikhail NM, Asem AA, Peter MvB (2015). Quantitative assessment of the differential impacts of arbuscular and ectomycorrhiza on soil carbon cycling. The New phytologist 208 (1):280-293. doi:10.1111/nph.13447

Pampolino MF, Urushiyama T, Hatano R (2000). Detection of nitrate leaching through bypass flow using pan lysimeter, suction cup, and resin capsule. Soil science and plant nutrition (Tokyo) 46 (3):703-711. doi:10.1080/00380768.2000.10409135

Panhwar QA, Ali A, Naher UA, Memon MY (2019). Chapter 2 - Fertilizer management strategies for enhancing nutrient use efficiency and sustainable wheat production. In: Chandran S, Unni MR, Thomas S (eds) Organic farming. Woodhead Publishing, pp 17-39. doi:https://doi.org/10.1016/B978-0-12-813272-2.00002-1

Parihar M, Meena V, Mishra P, Rakshit A, Choudhary M, Yadav R, Rana K, Bisht J (2019) Arbuscular mycorrhiza: a viable strategy for soil nutrient loss reduction. Archives of Microbiology 201 (6):723-735. doi:10.1007/s00203-019-01653-9

Paul EA (2014). Soil Microbiology, Ecology and Biochemistry. Elsevier Science & Technology, Saint Louis

Pellegrino E, Öpik M, Bonari E, Ercoli L (2015). Responses of wheat to arbuscular mycorrhizal fungi: A meta-analysis of field studies from 1975 to 2013. Soil biology & biochemistry 84:210-217. doi:10.1016/j.soilbio.2015.02.020

Qian P, Schoenau JJ (2002). Practical applications of ion exchange resins in agricultural and environmental soil research. Canadian journal of soil science 82 (1):9-21. doi:10.4141/S00-091

Ram AN, Asja B, Thomas A (2000). Analysis of phosphate acquisition efficiency in different Arabidopsis accessions. Plant physiology (Bethesda) 124 (4):1786-1799. doi:10.1104/pp.124.4.1786

Ramos-Zapata JA, Marrufo-Zapata D, Guadarrama P, Carrillo-Sánchez L, Hernández-Cuevas L, Caamal-Maldonado A (2012). Impact of weed control on arbuscular mycorrhizal fungi in a tropical agroecosystem: a long-term experiment. Mycorrhiza 22 (8):653-661. doi:10.1007/s00572-012-0443-1

Rillig M, Ramsey P, Gannon J, Mummey D, Gadkar V, Kapulnik Y (2008). Suitability of mycorrhiza-defective mutant/wildtype plant pairs (Solanum lycopersicum L. cv Micro-Tom) to

address questions in mycorrhizal soil ecology. Plant and Soil 308 (1):267-275. doi:10.1007/s11104-008-9629-x

Rillig MC, Mardatin NF, Leifheit EF, Antunes PM (2010). Mycelium of arbuscular mycorrhizal fungi increases soil water repellency and is sufficient to maintain water-stable soil aggregates. Soil biology & biochemistry 42 (7):1189-1191. doi:10.1016/j.soilbio.2010.03.027

Rillig MC, Mummey DL (2006). Mycorrhizas and soil structure. New Phytologist 171 (1):41-53. doi:10.1111/j.1469-8137.2006.01750.x

Rillig MC, Mummey DL, Ramsey PW, Klironomos JN, Gannon JE (2006). Phylogeny of arbuscular mycorrhizal fungi predicts community composition of symbiosis-associated bacteria. FEMS microbiology ecology 57 (3):389-395. doi:10.1111/j.1574-6941.2006.00129.x

Sharpley A, Jarvie HP, Buda A, May L, Spears B, Kleinman P (2013). Phosphorus legacy: overcoming the effects of past management practices to mitigate future water quality impairment. Journal of Environmental Quality 42 (5):1308-1326. doi:10.2134/jeq2013.03.0098

Smith ME, Facelli JM, Cavagnaro TR (2018). Interactions between soil properties, soil microbes and plants in remnant-grassland and old-field areas: a reciprocal transplant approach. Plant and soil 433 (1-2):127-145. doi:10.1007/s11104-018-3823-2

Smith SE, Smith FA (2011). Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annual Review of Plant Biology, vol 62. doi:10.1146/annurev-arplant-042110-103846

Taylor DL, Walters WA, Lennon NJ, Bochicchio J, Krohn A, Caporaso JG, Pennanen T (2016) Accurate estimation of fungal diversity and abundance through improved lineage-specific primers optimized for illumina amplicon sequencing. Applied and environmental microbiology 82 (24):7217-7226. doi:10.1128/AEM.02576-1610.1128/AEM.02576-16.

Tran CTK, Watts-Williams SJ, Smernik RJ, Cavagnaro TR (2020). Effects of plant roots and arbuscular mycorrhizas on soil phosphorus leaching. Science of the Total Environment 722. doi:10.1016/j.scitotenv.2020.137847

van der Heijden MGA (2010). Mycorrhizal fungi reduce nutrient loss from model grassland ecosystems. Ecology 91 (4):1163-1171. doi:10.1890/09-0336.1

Verbruggen E, Kiers ET, Bakelaar PNC, Röling WFM, van der Heijden MGA (2012). Provision of contrasting ecosystem services by soil communities from different agricultural fields. Plant and Soil 350 (1-2):43-55. doi:10.1007/s11104-011-0828-5

Vierheilig H, Coughlan AP, Wyss U, Piché Y (1998). Ink and vinegar, a simple staining technique for arbuscular mycorrhizal fungi. Applied and Environmental Microbiology 64 (12):5004-5007

Watts-Williams SJ, Cavagnaro TR (2015). Using mycorrhiza-defective mutant genotypes of non-legume plant species to study the formation and functioning of arbuscular mycorrhiza: a review. Mycorrhiza 25 (8):587-597. doi:10.1007/s00572-015-0639-2

Wheal MS, Fowles TO, Palmer LT (2011). A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of plant essential elements. Analytical Methods 3 (12):2854-2863. doi:10.1039/C1AY05430A

Wheeler RD, Baon JB, Alston AM, Smith SE (1992). Phosphorus efficiency of three cereals as related to indigenous mycorrhizal infection. Australian journal of agricultural research 43 (3):479. doi:10.1071/AR9920479

White PJ (2009). Efficiency of soil and fertilizer phosphorus use: reconciling changing concepts of soil phosphorus behaviour with agronomic information. Ex. Agric., vol 45. doi:10.1017/S0014479708007138

Wiedenfeld B, Enciso J (2008). Sugarcane responses to irrigation and nitrogen in semiarid south Texas. Agronomy journal 100 (3):665-671. doi:10.2134/agronj2007.0286

Wright ES (2016). Using DECIPHER v2.0 to analyze big biological sequence data in R. The R journal 8 (1):352. doi:10.32614/rj-2016-025

Xia H-Y, Zhao J-H, Sun J-H, Bao X-G, Christie P, Zhang F-S, Li L (2013). Dynamics of root length and distribution and shoot biomass of maize as affected by intercropping with different companion crops and phosphorus application rates. Field crops research 150:52-62. doi:10.1016/j.fcr.2013.05.027

Xu J, Liu S, Song S, Guo H, Tang J, Yong JWH, Ma Y, Chen X (2018). Arbuscular mycorrhizal fungi influence decomposition and the associated soil microbial community under different soil phosphorus availability. Soil biology & biochemistry 120:181-190. doi:10.1016/j.soilbio.2018.02.010

Zhang H, Liu T, Wang Y, Tang M (2019). Exogenous arbuscular mycorrhizal fungi increase soil organic carbon and change microbial community in poplar rhizosphere. Plant, soil and environment 65 (No. 3):152-158. doi:10.17221/2/2019-PSE

Zhang S, Yu X, Rillig M (2020). Arbuscular mycorrhiza contributes to the control of phosphorus loss in paddy fields. Plant and Soil 447 (1-2):623-636. doi:10.1007/s11104-019-04394-2

Chapter 5-A study on the phosphorus uptake and storage in external mycelium of arbuscular mycorrhizal fungi

This work contained in this chapter did not develop into a major theme in this project, some important results were found and so are presented herein.

Abstract

Despite their importance in plant phosphorus (P) uptake, there is a lack of research on the P metabolism of the external mycelium of arbuscular mycorrhizal fungi (AMF). Therefore, the objective of this study was to characterize the P metabolism and storage of inorganic polyphosphate (PolyP) in AMF hyphae. In this study, *Medicago truncatula* (Barrel medic) and *Allium ampeloprasum* (leek) were inoculated with the AMF *Rhizophagus irregularis* using a hyphae-root compartmented growth system. Extraradical hyphae were harvested after eight weeks. Mycorrhizal colonization increased P acquisition of the host plant species. Mycorrhizal *M. truncatula* pots had a higher external hyphal biomass than that of *A. ampeloprasum*. ^{31}P nuclear magnetic resonance (NMR) was used to investigate chemical speciation of P in the mycorrhizal external mycelium. The ^{31}P NMR spectra for the two mycorrhizal hyphae samples harvested from the leek and medic pots were very similar and PolyP peaks were clearly identified in these hyphal samples.

5.1 Introduction

Arbuscular mycorrhizal fungi (AMF) are a widespread group of soil fungi that establish a symbiotic association with the roots of most terrestrial plant species (Smith and Smith 2011). The AMF receive a supply of fixed carbon from the host plant (Berta et al. 2000); in return, AMF facilitate plant nutrient, especially phosphorus (P), acquisition (Bucher 2007; Smith et al. 2004). The network of mycorrhizal hyphae can extend more than 10 cm beyond the root surface (Jakobsen et al. 1992), allowing the roots access a larger soil volume and thus enhancing plant nutrient uptake (Bowles et al. 2016). Up to 90% of plant P can be acquired via AMF pathway (Cavagnaro et al. 2015).

The P absorbed by the external hyphal network is transferred to the internal hyphae and then to the host plants across a specialised membrane system (Smith et al. 2008). Following absorption as inorganic orthophosphate (Pi) from soil solution, AMF convert much of the P to PolyP, a linear polymer of phosphate linked by high-energy bonds (Moreno and Docampo 2013), for translocation along the mycelium (Callow et al. 1978; Solaiman et al. 1999). As PolyP plays a vital role in the transport, accumulation and transfer of P in AMF hyphal systems (Nowaki et al. 2010), various approaches has been used to detect the presence PolyP, including staining techniques (Kojima et al. 1998), radiotracer techniques (Schweiger et al. 1999), and enzymatic methods (Ezawa et al. 2004).

Nuclear magnetic resonance (NMR) spectroscopy is an analytical technique that is based on the analysis of the magnetic properties of the atomic nucleus (Abdi et al. 2014). This analysis approach is a powerful, nondestructive technique capable of achieving complete structural and conformational analysis of complex molecules, and quantitative analysis of complex mixtures (Smith and Blandford 1995). The advantage of this technique is that a wide range of compounds can be detected in a single experiment (Colquhoun and Lees 1998). For example, for NMR spectroscopy tuned to P, all the P-containing compounds should be similarly observable. The use of ^{31}P NMR spectroscopy has been successfully demonstrated for characterizing PolyP in the external mycelium of AMF (Rasmussen et al. 2000; Viereck et al. 2004). Due to the difficulty in collecting sufficient external hyphae biomass for NMR analysis, few *in vivo* ^{31}P NMR study of P metabolism in the mycelium of AMF have been reported. Moreover, there presently exists a lack of research on the comparison of PolyP storage in the external mycelium of AMF among different host plants.

In this experiment, *Medicago truncatula* (named medic hereafter) and *Allium ampeloprasum* (named leek hereafter) plants were inoculated with the AMF *Rhizophagus*

irregularis using a hyphae-root compartmented growth system. The aim of the present study was to compare plant growth, P plant uptake and the formation of external mycelium of this fungus. A ^{31}P NMR spectroscopy study was also included to identify the form in which P was present in the hyphae. The following plant growth and mycorrhizal development parameters were measured:

(i) mycorrhizal colonization, plant growth and P plant uptake;

(ii) external mycelium of *Rhizophagus irregularis* in the hyphal compartment;

(iii) chemical speciation of P in the mycorrhizal external mycelium of *Rhizophagus irregularis* using ^{31}P NMR spectroscopy.

5.2 Materials and methods

5.2.1 Experimental design

The experiment was conducted in a glasshouse at the University of Adelaide's Waite Campus (Adelaide, South Australia) from October to December 2018. Barrel medic and leek were used as host plants. There were 10 mycorrhizal plants and 10 non-mycorrhizal plants of each host plant species, giving a total of 40 plants.

All plants were grown in compartmented pots, following the method of Jasper et al. (1989), comprising an inner compartment in which roots were constrained and an outer compartment that only hyphae could access through a nylon mesh barrier that separated the inner and outer compartments (Figure 11). This system allowed separation and collection of external mycorrhizal hyphae. The root compartment was 80 mm height $\times$ 70 mm width and was filled with 600 g autoclaved soil:sand mixture (1:9 w/w).

The soil used was collected from the 0-10 cm layer of the University of Adelaide's Waite Campus Arboretum, South Australia. Soil was sieved to <2 mm to eliminate any coarse debris, autoclaved, dried and then mixed with the fine sand. The soil:sand mixture was mixed with 35 g of either a mycorrhizal inoculum or a non-mycorrhizal mock inoculum. The AMF inoculum was a mixture of dry soil, spores and external hyphae of *Rhizophagus irregularis* WFVAM10 (formerly named *Glomus intraradices*) and root fragment of *Trifolium subterraneum* L. (clover) cv. Mt Barket pot cultures. The control (mock) inoculum was a mixture of dry soil and root fragments that had not been inoculated with AMF.

The nylon mesh bag was placed in the centre of the hyphae compartment. The hyphae compartment was a 110 mm height and 120 mm diameter plastic pot, filled with 900 g autoclaved fine sand (see Figure 1 for more details about the growth system). Supplemental P was mixed with the sand of the hyphae compartment as $CaHPO_4$ powder at the rate of 50 mg P kg^{-1} sand.

Seeds of medic and leek were sterilised by shaking in 10% sodium hypochlorite, and then carefully rinsing under reverse osmosis (RO) water. Medic seeds were then plated onto moist filter paper, sealed, and incubated for three days at 4°C, then another four days at room temperature. Leek seeds were inoculated for 5 days at 25°C. One medic or five leek seedlings were sown directly into the root compartments of each pot as leek has a smaller size than medic.

Plants were grown in a controlled environment glasshouse with 14.5/9.5 h light/dark cycle; mean minimum and maximum temperature were 19.8°C and 26.5°C, respectively; mean minimum and maximum humidity were 37.3% and 65.2%, respectively. Plants were watered with RO water to a gravimetric soil moisture content of 10% every day, and once per week were nutritionally supplemented with 10 mL of a modified Long-Ashton solution that omitted P (Cavagnaro et al. 2001). Three weeks after transplanting, nitrogen was added at a rate of 40 mg N per pot as NH_4NO_3 solution to address the appearance of leaf nitrogen deficiency symptoms.

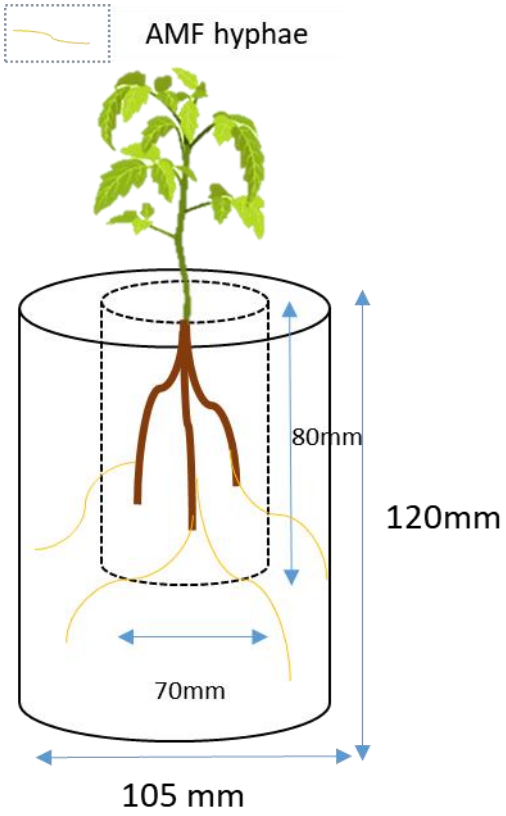

Figure 11. The hyphae-root compartmented growth system, consisting of the nylon mesh bag (having plants) and the outer hyphal compartment.

5.2.2 Plant biomass and external mycelium harvest

Eight weeks after transplanting, the shoots of plants were cut at the soil surface. The root compartment was separated from the hyphae compartment. Roots were collected and washed from the remaining soil and fresh root weight was determined. Subsamples of roots were cleared with 10% KOH (w/v) and stained with 5% ink in vinegar (Vierheilig et al. 1998) and root mycorrhizal colonization was determined using the gridline intersect method for 100 intersections (Giovannetti and Mosse 1980). The shoot and root were dried at 60^{o}C for 48 h and shoot and root dry weight determined. These plant materials were then ground to a fine powder. The P concentration of shoots, roots and hyphae were analysed using an Avio 200 ICP-OES (Perkin Elmer), following digestion with nitric acid and hydrochloric acid (Wheal et al. 2011).

The fine sand from the hyphae compartment was suspended in a one litre of RO water, and gently stirred by hand. After the sand particles settled, the suspended external mycelium was carefully collected using forceps. The suspension was then passed through a 38 µm sieve for a second hyphal collection. This procedure was repeated until the upper layer of the sand suspension was clear. The collected external mycelium was rinsed with RO water to remove any

remaining sand. The collected hyphae were immediately weighed and observed under the microscope (Nikon SMZ 745T). The hyphae sample from each pot was placed in 0.5 mL RO water and kept at 4°C until further analysis.

5.2.3 External mycelium analysis

The external mycelium samples of *Rhizophagus irregularis* were dried at 40^{o}C and ground using a small plastic mortar and pestle. The dried and ground hyphae samples were shaken with 0.25M NaOH and 0.05M Na_2EDTA, following the procedure of (Bowman and Moir 1993) for 16 hours. The crude extract was centrifuged for 10 minutes (1600g), then filtered (Whatman #42) to remove coarse particles. The filtered NaOH-EDTA extract was frozen in liquid nitrogen and freeze-dried for analysis by ^{31}P NMR spectroscopy. The freeze dry extract was ground, re-dissolved in 5 mL of deionized water and centrifuged for 20 minutes (1400g). The pH of the solution was adjusted to ensure a pH>13 using 10 M NaOH. The supernatant solution (3.5 mL) was transferred to a 10 mm NMR tube along with 0.3 mL of deuterium oxide (heavy water) and 0.1mL of a 6.0 g/L MDP solution. ^{31}P NMR spectra were acquired using a Varian INOVA 400 MHz NMR spectrometer (Varian, Palo Alto, CA) at 24^{o}C (^{31}P frequency of 161.9 MHz).

5.3 Results

5.3.1 Mycorrhizal colonization

Whereas roots of the plants in all non-mycorrhizal treatments were not colonized, the mycorrhizal colonization in roots of mycorrhizal medics and leeks were quite high, at 54 ± 6% and 80 ± 3%, respectively (expressed as root length colonized on average across all treatments).

5.3.2 Plant biomass and nutrient uptake

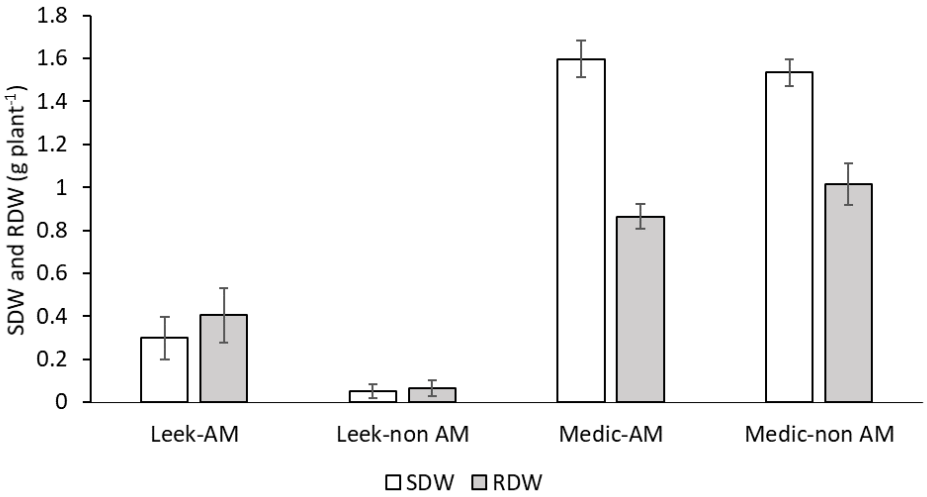

Figure 12. Mean of shoot (white bar) and root (grey bar) dry weight biomass of medics and leeks. Values are mean ± SE, n = 10.

After eight-weeks of growth, there were no differences in SDW and RDW between mycorrhizal and non-mycorrhizal medics, but the mycorrhizal leeks had a significantly higher SDW and RDW than non-mycorrhizal leeks (Figure 12).

Table 4. P concentration of shoot P concentration of shoot, root and hyphae tissues of medics and leeks. Values are mean ± SE, n=5.

P plant concentration (mg kg^{-1})	Leek-AM	Leek-non AM	Medic-AM	Medic-non AM
Shoot	1345 ± 94	1293 ± 58	2554 ± 202	2466 ± 215
Root	1599 ± 162	1097 ± 45	2579 ± 198	1323 ± 75
Hyphae	*	NA	1474 *	NA

* *Due to insufficient hyphae biomass, only samples of hyphae of medics was analysed for the P concentration.*

Mycorrhizal colonization increased the P concentration of root tissues of both host plants (Table 4). The P concentration in the external mycelium of the medics was 1474 mg P kg^{-1}, about equal to the P concentration of the mycorrhizal leek roots. Unfortunately, there was insufficient external hyphal biomass collected from the leek plants to determine the P concentration (Table 5).

5.3.3 Hyphae observation and hyphae biomass

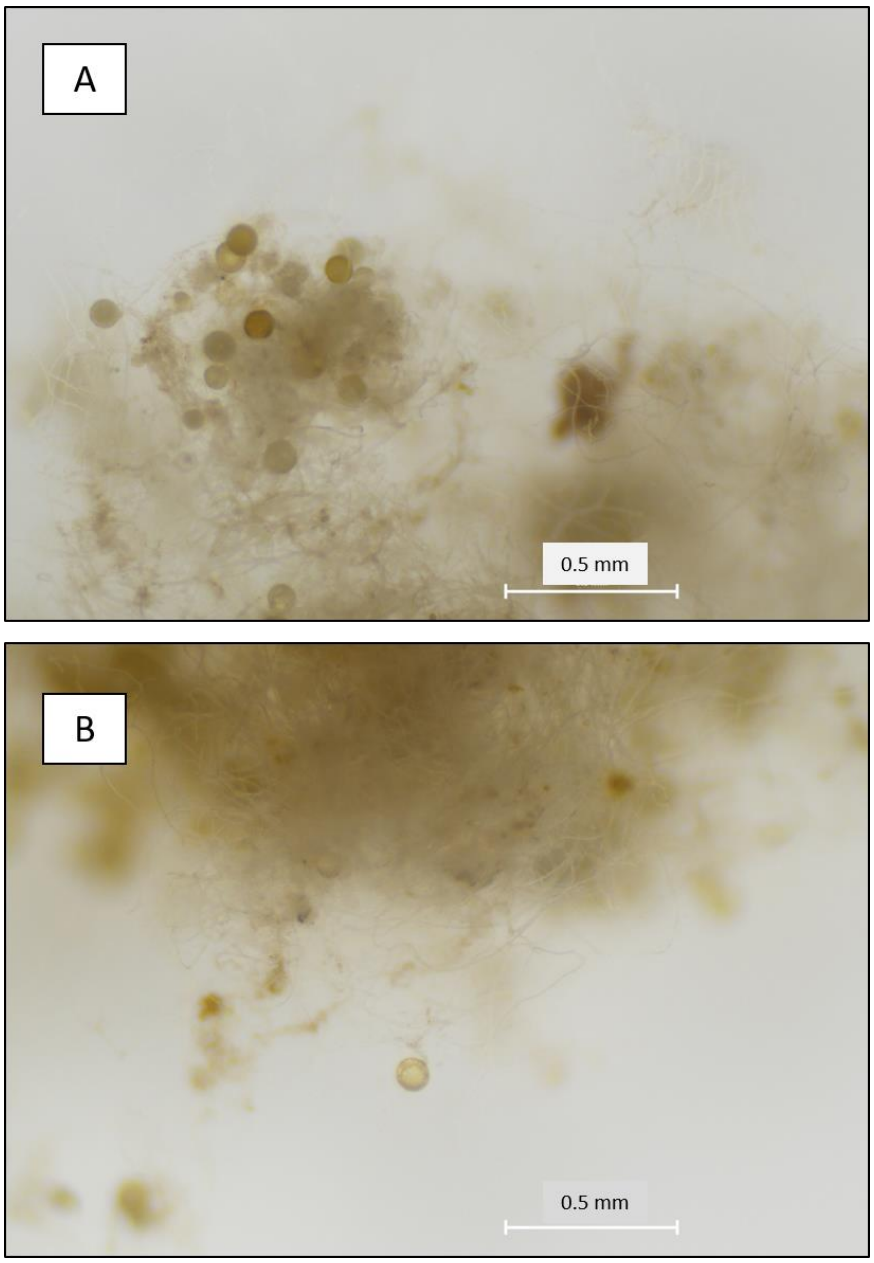

Figure 13. External mycelium formed by *Rhizophagus irregularis* harvested from the hyphae compartment of mycorrhizal leek (A) and medic (B) plants.

Table 5. External mycorrhizal hyphae biomass collected from medic and leek mycorrhizal pots

Pot number	Mycorrhizal hyphae biomass (fresh weight g)	
	Medic	Leek
1	0.354	0.167
2	0.196	0.116
3	0.348	0.049
4	0.276	0.102
5	0.287	0.069
6	0.301	0.220
7	0.265	0.100
8	0.03*	0.126
9	0.38	0.112
10	0.107	0.148
Total	2.544	1.209
Average	0.2544	0.1209

**This pot had less hyphae as the roots escaped to the hyphae compartment*

The appearance of extraradical mycorrhizal hyphae of *Rhizophagus irregularis* were similar for the medic and leek treatments (Figure 13). Spores of *Rhizophagus irregularis* were also evident after eight weeks growth. Despite the variation of the hyphae biomass among pots, the total fresh weight of hyphae collected from mycorrhizal medic pots were higher than that of mycorrhizal leek pots. The mean hyphae biomass harvested from medic pots was two times higher than those from leek.

5.3.4 ^{31}P NMR analysis

^{31}P NMR spectra for the mycorrhizal hyphae samples collected from leek and medic were very similar (Figure 14). The strongest signal at 5.5 ppm was identified as orthophosphate; two signals in the range 4.5-5.0 ppm were assigned as organic monoesters (almost certainly α- and β-glycerophosphate that come from alkaline hydrolysis of phospholipids in the NaOH-EDTA extract). There were two unknown peaks at ~1 ppm and ~3 ppm. A large peak at -21 ppm was assigned to P in the "middle" of PolyP chains, while P at the end of these chains are likely responsible for weak peaks at approximately -4.5 ppm. A small peak at -5 ppm is likely due to pyrophosphate.

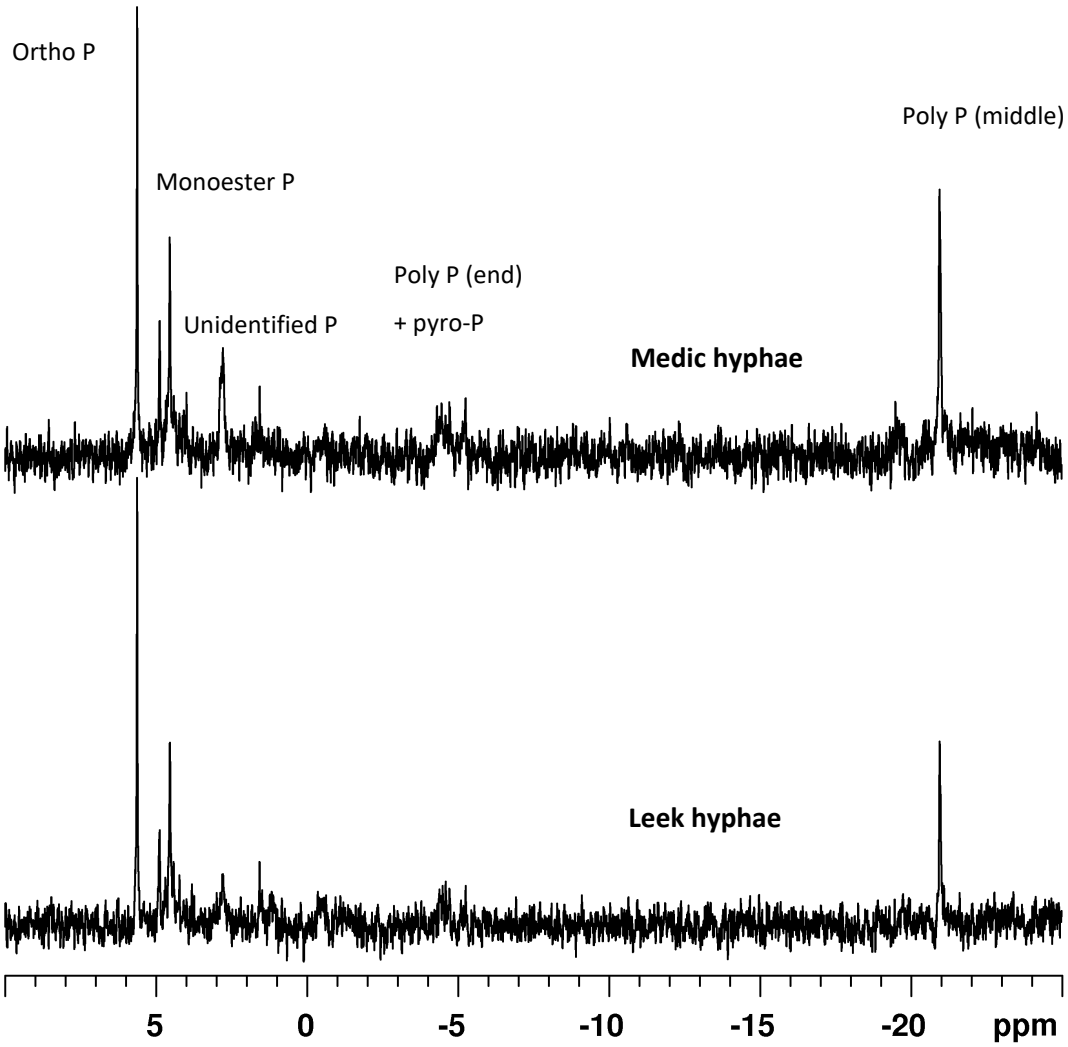

Figure 14. ^{31}P NMR spectra of NaOH-EDTA extracts of *Rhizophagus irregularis* mycelium of medic and leek pots.

5.4 Discussion

In this study, the mycorrhizal colonization of medic and leek plants by *Rhizophagus irregularis* were high, reflecting the high propensity of these two host plants species to form AM. The level of mycorrhizal colonization of medic is consistent with the previous study of Nguyen et al. (2019), in which the same medic variety, mycorrhizal fungal inoculum, soil mixture and soil P conditions were used. The impacts of mycorrhizal colonization on plant biomass of medic and leek were different; while there was no significant difference in plant biomass between mycorrhizal and non-mycorrhizal medic plants, mycorrhizal colonization resulted in a significant increase in plant biomass for leeks.

After eight weeks, *Rhizophagus irregularis* had clearly formed a network of external mycelium and clusters of spores were observed for both host plants. The formation of these external hyphal networks likely contributed to the higher P concentration in the mycorrhizal roots compared to the non-mycorrhizal roots of these plants. The hyphal biomass associated with mycorrhizal medic roots was two times higher than that of leek, despite there being only one medic plant per pot compared to the five leek plants per pot. This might be due to a higher root biomass of medics compared to leeks.

The P concentration in the hyphae was determined to ensure there was sufficient P in the mycorrhizal hyphae for NMR analysis (Silva Elipe 2003). The P concentration in mycorrhizal hyphae collected from medic pots was similar to the P concentration of the mycorrhizal leek roots, indicating the ability of P absorption and storage in the hyphae of this AMF. Unfortunately, due to the low amounts of hyphal biomass per pot, the P concentration of hyphae samples could only be carried out on a pooled sample.

Despite the difference in mycorrhizal colonization, plant biomass and P uptake between leek and medic as host plants, the ^{31}P NMR spectra for the two mycorrhizal hyphae samples were very similar. The presence of a large and distinctive peak at -21 ppm is consistent with the presence of substantial quantities of PolyP in the external mycorrhizal hyphae, as reported in previous studies of (Viereck et al. 2004; Rasmussen et al. 2000). The ^{31}P NMR spectra revealed a clear PolyP peak, however, the quality of peaks was not sufficient to enable qualification of the main P types present as well as total P in samples. In addition, two small peaks of PolyP and pyrophosphate were seen at approximately -4.5 ppm and -5 ppm, however, the terminal P peaks were too small to indicate the presence of PolyP molecules with different chain lengths.

These results confirm the formation of external mycorrhizal hyphae of *Rhizophagus irregularis* using a compartmented growth system with medic and leek as host plants. Medic can form a sufficient amount of external mycelium, which can be used for further study or analysis of mycorrhizal hyphae. Despite differences in mycorrhizal effects on plant P uptake and plant biomass, the presence and the storage of PolyP in the external mycorrhizal hyphal networks of *Rhizophagus irregularis* of these two host plants was nearly identical. The presence of the PolyP, and the P concentration in the mycorrhizal external hyphae, confirm the vital role of these hyphal systems in plant P uptake. These outcomes are important for further study on external mycorrhizal hyphae (e.g. hyphal architecture, morphology and nutrient uptake).

References

Abdi D, Cade-Menun BJ, Ziadi N, Parent LÉ (2014). Long-term impact of tillage practices and phosphorus fertilization on soil phosphorus forms as determined by ^{31}P nuclear magnetic resonance spectroscopy. Journal of environmental quality 43 (4):1431-1441. doi:10.2134/jeq2013.10.0424

Berta B, Philip EP, Yair S-H (2000). Carbon metabolism and transport in arbuscular mycorrhizas. Plant physiology (Bethesda) 124 (3):949-957. doi:10.1104/pp.124.3.949

Bowles TM, Barrios-Masias FH, Carlisle EA, Cavagnaro TR, Jackson Le (2016). Effects of arbuscular mycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. The Science of the total environment 566-567:1223-1234. doi:10.1016/j.scitotenv.2016.05.178

Bowman RA, Moir JO (1993). Basic EDTA as an extractant for soil organic phosphorus. Soil Science Society of America journal 57 (6):1516-1518. doi:10.2136/sssaj1993.03615995005700060020x

Bucher M (2007). Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. The New phytologist 173 (1):11-26. doi:10.1111/j.1469-8137.2006.01935.x

Callow JA, Capaccio LCM, Parish G, Tinker PB (1978). Detection and estimation of polyphosphate in vesicular and arbuscular mycorrhizas. New Phytologist 80 (1):125-134. doi:10.1111/j.1469-8137.1978.tb02272.x

Cavagnaro TR, Bender SF, Asghari HR, van der Heijden MGA (2015). The role of arbuscular mycorrhizas in reducing soil nutrient loss. Trends in Plant Science 20 (5):283-290. doi:10.1016/j.tplants.2015.03.004

Cavagnaro TR, Smith FA, Lorimer MF, Haskard KA, Ayling SM, Smith SE (2001). Quantitative development of Paris-type arbuscular mycorrhizas formed between Asphodelus fistulosus and Glomus coronatum. New Phytologist 149 (1):105-113. doi:doi:10.1046/j.1469-8137.2001.00001.x

Colquhoun LJ, Lees M (1998). Nuclear magnetic resonance spectroscopy. In: Ashurst PR, Dennis MJ (eds) Analytical methods of food authentication. Springer Science & Business Media,

Ezawa T, Timothy RC, Sally ES, Smith FA, Ryo O (2004). Rapid accumulation of polyphosphate in extraradical hyphae of an arbuscular mycorrhizal fungus as revealed by histochemistry and a polyphosphate kinase/Luciferase system. The New phytologist 161 (2):387-392. doi:10.1046/j.1469-8137.2003.00966.x

Giovannetti M, Mosse B (1980). Evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist 84:498-500

Jakobsen I, Abbott LK, Robson AD (1992). External hyphae of vesicular-arbuscular mycorrhizal fungi associated with Trifolium subterraneum L. 1. Spread of hyphae and phosphorus inflow into roots. The New Phytologist 120 (3):371-379. doi:10.1111/j.1469-8137.1992.tb01077.x

Jasper DA, Abbott LK, Robson AD (1989). Soil disturbance reduces the infectivity of external hyphae of vesicular- arbuscular mycorrhizal fungi. The New phytologist 112 (1):93-99. doi:10.1111/j.1469-8137.1989.tb00313.x

Kojima T, Hayatsu M, Saito M (1998). Intraradical hyphae phosphatase of the arbuscular mycorrhizal fungus, Gigaspora margarita. Biology and fertility of soils 26 (4):331-335. doi:10.1007/s003740050384

Moreno SNJ, Docampo R (2013). Polyphosphate and its diverse functions in host cells and pathogens. PLoS pathogens 9 (5):e1003230-e1003230. doi:10.1371/journal.ppat.1003230

Nguyen TD, Cavagnaro TR, Watts-Williams SJ (2019). The effects of soil phosphorus and zinc availability on plant responses to mycorrhizal fungi: a physiological and molecular assessment. Scientific reports 9 (1):14880-14813. doi:10.1038/s41598-019-51369-5

Nowaki H, Masatake M, Chiharu T, Ryo O, Mitsuru O, Tatsuhiro E (2010). Polyphosphate has a central role in the rapid and massive accumulation of phosphorus in extraradical mycelium of an arbuscular mycorrhizal fungus. The New phytologist 186 (2):285-289. doi:10.1111/j.1469-8137.2009.03168.x

Rasmussen N, Lloyd DC, Ratcliffe RG, Hansen PE, Jakobsen I (2000). ^{31}P NMR for the study of phosphorus metabolism and translocation in arbuscular mycorrhizal fungi. Plant and Soil 226 (1):245

Schweiger PF, Thingstrup I, Jakobsen I (1999). Comparison of two test systems for measuring plant phosphorus uptake via arbuscular mycorrhizal fungi. Mycorrhiza 8 (4):207-213. doi:10.1007/s005720050236

Silva Elipe MV (2003). Advantages and disadvantages of nuclear magnetic resonance spectroscopy as a hyphenated technique. Analytica Chimica Acta 497 (1):1-25. doi:10.1016/j.aca.2003.08.048

Smith ICP, Blandford DE (1995). Nuclear magnetic resonence spectrocospy. Analytical Chemistry 67 (12):509-518

Smith SE, Read DJ, Read DJ (2008). Mycorrhizal Symbiosis. Elsevier Science & Technology, San Diego

Smith SE, Smith FA (2011). Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annual Review of Plant Biology, vol 62. doi:10.1146/annurev-arplant-042110-103846

Smith SE, Smith FA, Iver J (2004). Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal phosphorus uptake pathway is not correlated with mycorrhizal responses in growth or total phosphorus uptake. The New phytologist 162 (2):511-524. doi:10.1111/j.1469-8137.2004.01039.x

Solaiman MZ, Ezawa T, Kojima T, Saito M (1999). Polyphosphates in intraradical and extraradical hyphae of an arbuscular mycorrhizal fungus, Gigaspora margarita. Applied and Environmental Microbiology 65 (12):5604

Viereck N, Poul Erik H, Iver J (2004). Phosphate pool dynamics in the arbuscular mycorrhizal fungus glomus intraradices studied by in vivo ^{31}P NMR spectroscopy. The New phytologist 162 (3):783-794. doi:10.1111/j.1469-8137.2004.01048.x

Vierheilig H, Coughlan AP, Wyss U, Piché Y (1998). Ink and vinegar, a simple staining technique for arbuscular mycorrhizal fungi. Applied and Environmental Microbiology 64 (12):5004-5007

Wheal MS, Fowles TO, Palmer LT (2011). A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-OES) analysis of plant essential elements. Analytical Methods 3 (12):2854-2863. doi:10.1039/C1AY05430A

Chapter 6- Conclusions

6.1 General conclusions

The overarching aim of the body of work presented in this thesis was to explore the effects of arbuscular mycorrhizal fungi (AMF) on soil phosphorus (P) leaching with an emphasis on P pools in the soil-plant-leachate system. Arbuscular mycorrhizas (AM) had consistently positive impacts on plant biomass, P uptake and fruit yield and nutrients across the experiments (Chapter 2, 3, 4 and 5). The evidence of inorganic polyphosphate (polyP) storage in the AMF external mycelium was identified in Chapter 5. Contrary to my initial hypothesis that AM can reduce P lost via leaching, an increase of soil P leached was observed in the mycorrhizal plant treatments in one experiment (Chapter 2). Importantly, the total P and dissolved organic carbon (DOC) were strongly correlated in this study. These results have implications for the interaction of AMF, roots and soil microbial communities, and soil P availability and leaching in non-sterilized soil systems. In Chapter 3, mycorrhizal colonization, plant growth, the chemical composition of P and the concentration of DOC leached, were different in two non-sterilized soil/sand mixes of contrasting texture. AM reduced P leaching in the sandier soil substrate, and this reduction was associated with greater DOC leached. These findings highlight the importance of soil texture in regulating AM effects on soil nutrient loss. The majority of P lost via leaching in this soil system was associated with the reactive P pool (Chapter 2 and 3). Mycorrhizal effects on soil P leaching were also extended to a field-based study (Chapter4). Under field soil conditions, mycorrhizal tomato plants without fertilizer had the same fruit yield and fruit nutrient concentrations as plants receiving fertilizer. Mycorrhizal effects on soil moisture, 16S bacterial community composition, and P loss via leaching were very small after a single cropping period, suggesting the need for future longer-term studies. Taken together, this work highlights the importance of AM in terms of plant yields, nutrient cycling and P leaching. This study also provides new insights into the complex interactions of roots, mycorrhizal roots and soil biota on nutrient leaching in soils.

6.1.1 Arbuscular mycorrhizas increase plant growth, P plant uptake and fruit biomass and nutrient concentrations

It is well established that AM have the capacity to increase plant biomass and nutrient uptake (Smith et al. 2011; Mathur and Vyas 1999). In this study, the mycorrhiza-defective tomato mutant, (*rmc*) and its wild-type progenitor (76R) were used to establish +/-AM treatments. The 76R plants experienced an increase in shoot biomass and plant P tissue content (Chapter 2). This

in an agreement with the meta-analysis of Watts-Williams and Cavagnaro (2014), indicating P plant tissue in the 76R genotype were generally higher than the *rmc* genotype. However, the mycorrhizal effects on these plant parameters were not consistent across all experiments in this study. There was a lack of mycorrhizal effects on tomato growth and nutrient uptake in the leaching experiments in Chapter 3 and Chapter 4. This was associated with a lower root length colonization of 76R plants in these experiments compared to that of Chapter 2. The mean of root length colonization of 76R plants across all treatment in Chapter 3 and Chapter 4 were 12.8 ± 1.3% and 13.8 ± 2.8%, respectively. These mycorrhizal colonization levels were significantly lower than that reported in Chapter 2, which were 37.5 ± 2.9%. These results agree with a review of Treseder (2013), who found that plant biomass and nutrient content rose when root length colonization increased. This finding highlights the importance of maintaining a sufficient level of mycorrhizal colonization root to enhance the mycorrhizal benefits on plant growth and nutrient uptake.

As AM can affect fruit metabolism (Zouari et al. 2014) and enhance plant reproductive growth (Jennifer et al. 2002), this symbiosis has been reported increase fruit yield and nutrient concentrations (Ziane et al. 2017). In the present study, a significant increase in tomato fruit yield and nutrient concentration was found for mycorrhizal plants compared to non-mycorrhizal plants, where additional P was not suppled as a fertiliser (Chapter 4). Of particular note was the fact that the 76R plants had the same fruit yield and nutrient concentrations, as the plants (be they mycorrhizal or not), where fertilizers were applied. Importantly, the greater fruit biomass of mycorrhizal plants was not associated with a higher plant biomass (SDW and RDW). This finding is consistent with an increase of fruit biomass (Bowles et al. 2016) and fruit nutrient concentrations (Cavagnaro et al. 2006) of this tomato mycorrhizal genotype under field conditions. This also highlights the beneficial effects of AM on the harvest index of mycorrhizal tomato plants, in which the allocation of plant resources to fruits over vegetative biomass, was likely prioritised in the mycorrhizal plants. Importantly, this positive mycorrhizal effect on fruit biomass was not observed when the available soil P level was high, and resulted in a reduction in mycorrhizal colonization of the roots (Chapter 4). This, again, indicates the important role of mycorrhizal root colonization under low P conditions on improving plant yields. Taken together, these findings suggest that AMF have a potentially important role to play in improving crop yields in situations where P fertiliser is not applied.

In the NMR study on the external mycorrhizal hyphae (Chapter 5), *Medicago truncatula* (Barrel medic) and *Allium ampeloprasum* (leek) were used as the host plants and grown in sterilized soil systems. The use of *Rhizophagus irregularis* inoculant resulted in a high level of

root mycorrhizal colonization and the formation of the external mycelium. Formation of AM led to a higher plant biomass and plant tissue P concentration of mycorrhizal plants compared to non-mycorrhizal plants. The presence of the polyphosphate peaks in the ^{31}P NMR spectra, highlights the important role of these fungal mycelium in P plant uptake and storage. Due to technical difficulties with this work, this avenue of investigation was not explored further, but the results were included herein for the sake of completeness.

6.1.2 Interaction of AMF, roots and soil microbes on the amount of soil P, P chemical composition and DOC leached

In this study, "no-plant" control treatments (i.e. plant-free controls) were included in the leaching experiments. This approach allows the comparisons of P dynamics between soils with and without roots, be they mycorrhizal or not. Roots can reduce soil P leaching by directly absorbing P and water, thereby decreasing the amount of P in the soil solution and leachate volume (Jiang et al. 2018). However, in this work, the plant treatments (mycorrhizal or not), had lower leachate volume but higher P and DOC concentration, compared to plant-free control treatments (Chapter 2 and 3). One explanation for this may be that roots might block water flow channels, leading to lower infiltration rates and hydraulic conductivity of planted-soils compare to bare soils (Leung et al. 2015). This situation may have been more pronounced given that the plants were grown in microcosms with a high root length density. Importantly, the influence of roots on increasing P and DOC leaching was observed in the two glasshouse leaching experiments (Chapter 2 and 3). Roots release exudates (e.g. organic acids and enzymes) to solubilize and mineralize mineral and complexed P forms (Roberts et al. 2020). Therefore, roots might mobilize more P into soil solution and influence soil physical properties, thereby increasing the amount of P available for leaching.

Nutrient cycling and leaching are complex processes and involve many soil microbes (bacteria and fungi) (Parihar et al. 2019). By using the tomato mutant genotypes, the effects of AM on soil P leaching were investigated in non-sterilized soil systems. The presence AMF together with the wider soil biota, revealed new insights about soil P leaching, especially the strong correlation between P and DOC leached reported in Chapter 2. Associations between P and DOC leached have been documented previously. For example, a greater amount of DOC leached has been recorded in high P soils compared to non-fertilized soils (Scott et al. 2015). The application of P to soil can stimulate DOC leaching due to the competition between P and DOC for anion binding sites in soils (Kang et al. 2011; Kalbitz et al. 2000). A significant positive correlation between P and DOC in soil leachate under excessive manure application has also been reported (Liu et al. 2019). In this study, the simultaneous P and DOC leached might be

explained due to the contribution of root exudation and soil microbial activities. Root exudation is considered as one of the main sources of C inputs into soils (Kalbitz et al. 2000). Moreover, the presence of AMF also contribute and influence C cycling in soil (Zhang et al. 2019; Rillig 2004). The presence of these low molecular organic acids in soils might decrease soil P adsorption (Lindegren and Persson 2010), thereby increasing the risk of P leaching. In addition, DOC is also a major energy source for soil microbial activity (Brailsford et al. 2019; Dong et al. 2012), which can be enhanced with AM (Artursson et al. 2006). The association of roots, AMF and other soil biota may also enhance the amount of available P in soil solution (Raghothama and Karthikeyan 2005; Roberts et al. 2020). It is clear that the influence of roots, AMF and soil microbial community on soil P and DOC leaching are complex and interdependent, indicating a need for further studies on this topic.

The amount of different P forms in soils and the ability of plants to acquire P, can be major factors in determining how plants affect leaching of soil P (Roberts et al. 2020). In this study, roots reduced the available soil P concentrations in the 0-10 cm and 10-25 cm soil layers in the microcosm experiment (Chapter 3), and (only) in the 0-10 cm layer in the field-based experiment (Chapter 4). Even though roots play a significant role in regulating soil P cycling and leaching, their impacts on soil available P are likely significant in surface soil layers where root density is high.

Understanding the chemical nature of P pools in soils is critical to efforts seeking to maximize plant P usage and to minimize soil P leaching (Stutter 2015). Phosphorus leached from soil can be in reactive P (dissolved PO_4^{3-}-P) or in unreactive forms (soluble and particulate organic P) (Pote et al. 2009). In this study, the majority of P leached was reactive P (65-75% total P leached), even though most of the soil P was unreactive P (Chapter 2 and 3). Importantly, a reduction of reactive P leached with the mycorrhizal plants in a sandy soil substrate was demonstrated in Chapter 3. This highlights the potential of AM in reducing P leaching in sandy soil, where P leaching is particularly problematic due to rapid soil water flow and low P adsorption (Nelson et al. 2005).

6.1.3 Mycorrhizal effects on soil P leaching depends on experimental conditions

Results from studies on AM effects on the loss of P via leaching have been somewhat inconsistent. While positive effects of AM on reducing P loss via leaching have been documented (van der Heijden 2010; Asghari and Cavagnaro 2011; Corkidi et al. 2011; Zhang et al. 2020), little or no effects have also been observed (Köhl and van der Heijden 2016; Duffková et al. 2019). Moreover, mycorrhizal effects on soil P loss can vary within a study e.g. an increase 20%

unreactive P, but no effects on dissolved organic P was seen in one study (Kohl et al. 2014), and there was an increase in total P loss in the first year but no effects in second year (Bender and van der Heijden 2015). In the present study, AM effects on soil P loss were also varied, depending upon experimental conditions. For example, in Chapter 2, AM consistently increased P leaching at two levels of P fertiliser application. These increases were attributed to higher P mobility in soils due to root exudation and soil microbial activities. In Chapter 3, there was a reduction of P loss in the mycorrhizal treatments in the sandy soil substrate, but no mycorrhizal effects in the fine soil substrate, even though the same AMF inoculant and host plants were used. These contrasting findings highlight the need for further work of this nature.

AMF can influence soil microbial diversity (Marschner et al. 2001; Rillig et al. 2006b). Therefore, a 16S sequencing study was included in the field leaching experiment to investigate the interaction between AMF, soil bacterial community and P leaching. However, soil bacterial community composition between mycorrhizal and non-mycorrhizal treatments were not significantly different. This may be because the duration of the experiment was not sufficient for detectable shifts in the soil microbiome to occur (Chapter 4). This result warrants further detailed investigation in a long-term experiment.

AMF can improve soil aggregation (Rillig and Mummey 2006) and soil water holding capacity (Cavagnaro et al. 2006; Augé 2004). Therefore, AMF may reduce soil nutrient loss by affecting soil moisture dynamics in the root zone and reducing the leachate volume (Cavagnaro et al. 2015). However, AM did not significantly affect the leachate volume in two the experiments in Chapter 2 and Chapter 3. This is in agreement with previous mycorrhizal leaching experiments (Asghari and Cavagnaro 2012; van der Heijden 2010). This result indicates that the amount of water leached and soil moisture might not contribute to the differences in the amount of nutrient leached in this soil system.

6.1.4 Recommended approaches for the field-based study of mycorrhizal effects on P leaching

To my knowledge, the work presented in Chapter 4 is the first field-based study on mycorrhizal effects on soil P leaching. This experiment presents some potential approaches to overcome the field-based study challenges. For example, anion-exchange resin membranes can be used as a reliable tool to examine the soil P leaching with minimal soil disturbance. Additionally, the mycorrhiza-defective tomato genotype (*rmc*) and its mycorrhizal wild-type progenitor (76R), were used to establish mycorrhizal and non-mycorrhizal treatments without the need to sterilize soils. Importantly, there were no significant differences in root biomass between these

tomato genotypes in the leaching experiments (Chapter 2, 3 and 4). This provides for a valid comparison of AM impacts on soil P loss via leaching without the confounding effects of plant size asymmetry. Moreover, it is suggested that a plant-free control treatment should also be included in such studies (as was the case here).

6.2 Future research directions

In undertaking this research, a number of avenues for potential future research have been identified, as follows:

It is clear that there are complex interactions between AMF, roots and the wider soil biota. These interactions are likely to have an impact on soil P cycling, and loss via leaching. Therefore, it is recommended that future research should focus on investigating the precise mechanisms of how roots (e.g. rhizosphere pH, organic exudation, phosphatase excretion) and soil microbial community (e.g. phosphate solubilizing bacteria) change between mycorrhizal and non-mycorrhizal plants, and how these changes influence soil P availability and P leaching.

Due to time limitations, the field-based study on mycorrhizal effects on P leaching was conducted over a single field season. Given that P moves slowly in soil, and mycorrhizal impacts on soil physical, chemical and biological properties take time to develop, it is suggested that a long-term field study be conducted to investigate AM impacts on these factors and soil nutrient leaching. Moreover, the reaction between soil particles and P may be strong in some soil systems; this impacts the amount of P leached, and therefore measures of P sorption and phosphorus buffering index (PBI) could be taken into account in future studies of soil P leaching.

AM might impact soil physical properties, such as soil aggregation and soil porosity, which in turn may impact leachate volume and the amount of nutrient leached (Chapter 2 and 3). This speculation could be further assessed by using non-destructive imaging technique such as computed tomography scanning. In addition, there was a strong relationship between DOC and P leached, and aspects of composition of the DOC leached could be examined in greater analytical detail e.g. measuring polyvalent cations in the leachate to confirm the linkages between plant-excreted DOC and these cations, and/or the chemical nature of the leached DOC (e.g. plant root exudates) (Chapter 2).

The present study used a single soil type (a fine sandy loam), noting the addition of sand in Chapter 3. It is, therefore, recommended that mycorrhizal effects on nutrient loss should be examined in soils with differing texture and P binding capacity. Similarly, it would be beneficial to undertake such experiments with different crops and AMF species.

References

Abdi D, Cade-Menun BJ, Ziadi N, Parent LÉ (2014). Long-term impact of tillage practices and phosphorus fertilization on soil phosphorus forms as determined by ^{31}P Nuclear magnetic resonance spectroscopy. Journal of environmental quality 43 (4):1431-1441. doi:10.2134/jeq2013.10.0424

Adesemoye AO, Kloepper JW (2009). Plant-microbes interactions in enhanced fertilizer-use efficiency. Applied Microbiology and Biotechnology 85 (1):1-12. doi:10.1007/s00253-009-2196-0

Aliasgharzad N, Neyshabouri MR, Salimi G (2006). Effects of arbuscular mycorrhizal fungi and *Bradyrhizobium japonicum* on drought stress of soybean. Biológia 61 (S19):S324-S328. doi:10.2478/s11756-006-0182-x

Allen MF (2011). Linking water and nutrients through the vadose zone: A fungal interface between the soil and plant systems. Journal of Arid Land 3 (3):155-163. doi:10.3724/SP.J.1227.2011.00155

Ames RN, Reid CPP, Ingham ER (1984). Rhizosphere bacterial population responses to root colonization by a vesicular-arbuscular mycorrhizal fungus. The New phytologist 96 (4):555-563

Andreas N, Anne KC (2006). Selective removal of DNA from dead cells of mixed bacterial communities by use of ethidium monoazide. Applied and Environmental Microbiology 72 (3):1997-2004. doi:10.1128/AEM.72.3.1997-2004.2006

Artursson V, Finlay RD, Jansson JK (2006). Interactions between arbuscular mycorrhizal fungi and bacteria and their potential for stimulating plant growth. Environmental microbiology 8 (1):1-10. doi:10.1111/j.1462-2920.2005.00942.x

Asghari HR, Cavagnaro TR (2011). Arbuscular mycorrhizas enhance plant interception of leached nutrients. Functional Plant Biology 38 (3):219-226. doi:10.1071/FP10180

Asghari HR, Cavagnaro TR (2012). Arbuscular mycorrhizas reduce nitrogen loss via leaching Ploss one 7 (1)

Asghari HR, Chittleborough DJ, Smith FA, Smith SE (2005). Influence of arbuscular mycorrhizal (AM) symbiosis on phosphorus leaching through soil cores. Plant and Soil 275 (1):181-193. doi:10.1007/s11104-005-1328-2

Atul-Nayyar A, Hamel C, Hanson K, Germida J (2009). The arbuscular mycorrhizal symbiosis links N mineralization to plant demand. Mycorrhiza 19 (4):239-246. doi:10.1007/s00572-008-0215-0

Augé RM (2004). Arbuscular mycorrhizae and soil/plant water relations. Canadian Journal of Soil Science 84 (4):373-381. doi:10.4141/S04-002

Baker JL, Campbell KL, Johnson HP, Hanway JJ (1975). Nitrate, phosphorus and sulfate in subsurface drainage water. Journal of Environmental Quality 4:406-412

Barea J-M, Azcón R, Azcón-Aguilar C (2002).Mycorrhizosphere interactions to improve plant fitness and soil quality. Antonie van Leeuwenhoek 81 (1):343-351. doi:10.1023/A:1020588701325

Barker SJ, Stummer B, Gao L, Dispain I, O'Connor PJ, Smith SE (1998a). A mutant in *Lycopersicon esculentum* Mill. with highly reduced VA mycorrhizal colonization: isolation and preliminary characterisation. The Plant Journal 15 (6):791-797. doi:doi:10.1046/j.1365-313X.1998.00252.x

Bender SF, Conen F, Van der Heijden MGA (2014). Mycorrhizal effects on nutrient cycling, nutrient leaching and N2O production in experimental grassland. Soil Biology and Biochemistry 80 (Supplement C):283-292. doi:https://doi.org/10.1016/j.soilbio.2014.10.016

Bender SF, van der Heijden MGA (2015). Soil biota enhance agricultural sustainability by improving crop yield, nutrient uptake and reducing nitrogen leaching losses. Journal of Applied Ecology 52 (1):228-239. doi:10.1111/1365-2664.12351

Berta B, Philip EP, Yair S-H (2000). Carbon metabolism and transport in arbuscular mycorrhizas. Plant physiology (Bethesda) 124 (3):949-957. doi:10.1104/pp.124.3.949

Bolan NS (1991). A critial review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant and Soil 134:189-207

Bolan NS, Robson AD, Barrow NJ (1987). Effect of vesicular-arbuscular mycorrhiza on the availibility of iron phosphates to plants. Plant and Soil 99:401-410

Bowles TM, Barrios-Masias FH, Carlisle EA, Cavagnaro TR, Jackson LE (2016). Effects of arbuscular mycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions. The Science of the total environment 566-567:1223-1234. doi:10.1016/j.scitotenv.2016.05.178

Bowles TM, Jackson LE, Cavagnaro TR (2017a). Mycorrhizal fungi enhance plant nutrient acquisition and module nitrogen loss with variable water regimes. Global Change Biology. doi:10.1111/gcb.13884

Bowles TM, Jackson LE, Loeher M, Cavagnaro TR, Nuñez M (2017b). Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. The Journal of applied ecology 54 (6):1785-1793. doi:10.1111/1365-2664.12815

Bowman RA, Moir JO (1993). Basic EDTA as an extractant for soil organic phosphorus. Soil Science Society of America journal 57 (6):1516-1518. doi:10.2136/sssaj1993.03615995005700060020x

Brailsford FL, Glanville HC, Golyshin PN, Johnes PJ, Yates CA, Jones DL (2019). Microbial uptake kinetics of dissolved organic carbon (DOC) compound groups from river water and sediments. Scientific reports 9 (1):11229-11211. doi:10.1038/s41598-019-47749-6

Brookes PC, Heckrath G, Smet JD, Hofman G, Vanderdeelen J (1997). Losses of phosphorus indrainage water. In: Tunney H, Carton OT, Brookes PC, Johnston AE (eds) Phosphorus loss from soil to water, CAB International Wallingford, pp 253-271

Bucher M (2007). Functional biology of plant phosphate uptake at root and mycorrhiza interfaces. The New phytologist 173 (1):11-26. doi:10.1111/j.1469-8137.2006.01935.x

Butterly C, McNeill A, Baldock J, Marschner P (2011). Rapid changes in carbon and phosphorus after rewetting of dry soil. Biology and Fertility of Soils 47 (1):41-50. doi:10.1007/s00374-010-0500-x

Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature methods 13 (7):581-583. doi:10.1038/nmeth.3869

Callow JA, Capaccio LCM, Parish G, Tinker PB (1978). Detection and estimation of polyphosphate in vesicular and arbuscular mycorrhizas. New Phytologist 80 (1):125-134. doi:10.1111/j.1469-8137.1978.tb02272.x

Cameron KC, Di HJ, Moir JL (2013). Nitrogen losses from the soil/plant system: a review. Annals of applied biology 162 (2):145-173. doi:10.1111/aab.12014

Carpio LA, Davies FT, Jr., Arnold MA (2005). Arbuscular mycorrhizal fungi, organic and inorganic controlled-release fertilizers: effect on growth and leachate of container-grown bush morning glory (Ipomoea carnea ssp. fistulosa) under high production temperatures. Journal of the American Society for Horticultural Science 130 (1):131-139. doi:10.21273/JASHS.130.1.131

Carstensen J, Andersen JH, Gustafsson BG, Conley DJ (2014). Deoxygenation of the baltic sea during the last century. Proceedings of the National Academy of Sciences of the United States of America 111 (15):5628-5633. doi:10.1073/pnas.1323156111

Cavagnaro TR (2008). The role of arbuscular mycorrhizas in improving plant zinc nutrition under low soil zinc concentrations: A review. Plant and Soil 304 (1-2):315-325. doi:10.1007/s11104-008-9559-7

Cavagnaro TR (2016). Soil moisture legacy effects: Impacts on soil nutrients, plants and mycorrhizal responsiveness. Soil biology & biochemistry 95:173-179. doi:10.1016/j.soilbio.2015.12.016

Cavagnaro TR, Bender SF, Asghari HR, van der Heijden MGA (2015). The role of arbuscular mycorrhizas in reducing soil nutrient loss. Trends in Plant Science 20 (5):283-290. doi:10.1016/j.tplants.2015.03.004

Cavagnaro TR, Jackson LE, Scow KM, Hristova KR (2007). Effects of arbuscular mycorrhizas on ammonia oxidizing bacteria in an arganic farm soil. Microbial ecology 54 (4):618-626. doi:10.1007/s00248-007-9212-7

Cavagnaro TR, Jackson LE, Six J, Ferris H, Goyal S, Asami D, Scow KM (2006). Arbuscular mycorrhizas, microbial communities, nutrient availability, and soil aggregates in organic tomato production. Plant and Soil 282 (1):209-225. doi:10.1007/s11104-005-5847-7

Cavagnaro TR, Smith FA, Hay G, Carne-Cavagnaro VL, Smith SE (2004). Inoculum type does not affect overall resistance of an arbuscular mycorrhiza-defective tomato mutant to colonisation but inoculation does change competitive interactions with wild-type tomato. New Phytologist 161 (2):485-494. doi:doi:10.1111/j.1469-8137.2004.00967.x

Cavagnaro TR, Smith FA, Lorimer MF, Haskard KA, Ayling SM, Smith SE (2001). Quantitative development of Paris-type arbuscular mycorrhizas formed between *Asphodelus fistulosus* and *Glomus coronatum*. New Phytologist 149 (1):105-113. doi:doi:10.1046/j.1469-8137.2001.00001.x

Cavagnaro TR, Smith FA, Smith SE, Jakobsen I (2005). Functional diversity in arbuscular mycorrhizas: Exploitation of soil patches with different phosphate enrichment differs among fungal species. Plant, Cell and Environment 28 (5):642-650. doi:10.1111/j.1365-3040.2005.01310.x

Chen GC, He ZL, Stoffella PJ, Yang XE, Yu S, Calvert D (2006). Use of dolomite phosphate rock (DPR) fertilizers to reduce phosphorus leaching from sandy soil. Environmental pollution (1987) 139 (1):176-182. doi:10.1016/j.envpol.2004.12.016

Clark RB, Zeto SK (2008). Mineral acquisition by arbuscular mycorrhizas plants. Journal of Plant Nutrition 23 (7):867-902. doi: 10.1080/01904160009382068

Colquhoun LJ, Lees M (1998). Nuclear magnetic resonance spectroscopy. In: Ashurst PR, Dennis MJ (eds) Analytical methods of food authentication. Springer Science & Business Media,

Colwell JD (1963). The estimation of phosphorus fertiliser requirements of wheat in southern New South Wales Australian Journal of Experimental Agriculture:190-197

Cordell D, Drangert J-O, White S (2009a). The story of phosphorus: global food security and food for thought. Global Environmental Change 19 (2):292-305. doi:10.1016/j.gloenvcha.2008.10.009

Corkidi L, Merhaut DJ, Allen EB, Downer J, Bohn J, Evans M (2011). Effects of mycorrhizal colonization on nitrogen and phosphorus leaching from nursery containers. HortScience 46 (11):1472-1479

Dai Z, Liu G, Chen H, Chen C, Wang J, Ai S, Wei D, Li D, Ma B, Tang C, Brookes PC, Xu J (2020) Long-term nutrient inputs shift soil microbial functional profiles of phosphorus cycling in diverse agroecosystems. The ISME Journal 14 (3):757-770. doi:10.1038/s41396-019-0567-9

David DD, Philip EP, Yair S-H (2000). Application of in vitro methods to study carbon uptake and transport by AM fungi. Plant and soil 226 (2):255-261. doi:10.1023/A:1026466802354

Djodjic F, Bergström L, Grant C (2005). Phosphorus management in balanced agricultural systems. Soil Use and Management 21:94-101. doi:10.1111/j.1475-2743.2005.tb00413.x

Djodjic F, Börling K, Bergström L (2004). Phosphorus leaching in relation to soil type and soil phosphorus content. Journal of Environmental Quality 33 (2):678-684. doi:10.2134/jeq2004.6780

Dong L, Jonathan OS, Pascal ES, Shahjahan A, Mazahirali A, Mohammed SA, Stephanie K, Christiane H-J, Jörg ED (2012). Dissolved organic carbon influences microbial community composition and diversity in managed aquifer recharge systems. Applied and Environmental Microbiology 78 (19):6819-6828. doi:10.1128/AEM.01223-12

Dong W, Qin J, Li J, Zhao Y, Nie L, Zhang Z (2011). Interactions between soil water content and fertilizer on growth characteristics and biomass yield of Chinese white poplar (Populus

tomentosa Carr.) seedlings. Soil science and plant nutrition (Tokyo) 57 (2):303-312. doi:10.1080/00380768.2010.549445

Drerup P, Brueck H, Eticha D, Scherer HW (2019). NDVI-based estimates of evapotranspiration of winter wheat indicate positive effects of N fertilizer application on agronomic water-use efficiency. Journal of agronomy and crop science (1986) 206 (1):1-12. doi:10.1111/jac.12364

Duffková R, Fučík P, Jurkovská L, Janoušková M (2019). Experimental evaluation of the potential of arbuscular mycorrhiza to modify nutrient leaching in three arable soils located on one slope. Applied soil ecology : a section of Agriculture, ecosystems & environment 143:116-125. doi:10.1016/j.apsoil.2019.06.001

El Maaloum S, Elabed A, Alaoui-Talibi ZE, Meddich A, Filali-Maltouf A, Douira A, Ibnsouda-Koraichi S, Amir S, El Modafar C (2020). Effect of arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria consortia associated with phospho-compost on phosphorus solubilization and growth of tomato Seedlings (*Solanum lycopersicum* L.). Communications in soil science and plant analysis 51 (5):622-634. doi:10.1080/00103624.2020.1729376

Ezawa T, Timothy RC, Sally ES, Smith FA, Ryo O (2004). Rapid accumulation of polyphosphate in extraradical hyphae of an arbuscular mycorrhizal fungus as revealed by histochemistry and a polyphosphate kinase/luciferase system. The New phytologist 161 (2):387-392. doi:10.1046/j.1469-8137.2003.00966.x

Frossard E, Condron LM, Oberson A, Sinaj S, Fardeau JC (2000). Processes governing phosphorus availability in temperate soils. Journal of Environmental Quality 29 (1):15-23. doi:10.2134/jeq2000.00472425002900010003x

Gianinazzi S, Trouvelot A, Gianinazzi-Pearson V (1990). Conceptual approaches for the rational use of VA endomycorrhizae in agriculture: Possibilities and limitations. Agriculture, ecosystems & environment 29 (1):153-161. doi:10.1016/0167-8809(90)90269-J

Giovannetti M, Mosse B (1980). Evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist 84:498-500

Gosling P, Mead A, Proctor M, Hammond JP, Bending GD (2013). Contrasting arbuscular mycorrhizal communities colonizing different host plants show a similar response to a soil phosphorus concentration gradient. The New phytologist 198 (2):546-556. doi:10.1111/nph.12169

Grossman JD, Rice KJ (2012). Evolution of root plasticity responses to variation in soil nutrient distribution and concentration. Evolutionary applications 5 (8):850-857. doi:10.1111/j.1752-4571.2012.00263.x

Gui H, Hyde K, Xu J, Mortimer P (2017). Arbuscular mycorrhiza enhance the rate of litter decomposition while inhibiting soil microbial community development. Scientific reports 7 (1):42184-42184. doi:10.1038/srep42184

Harmanto, Salokhe VM, Babel MS, Tantau HJ (2005). Water requirement of drip irrigated tomatoes grown in greenhouse in tropical environment. Agricultural Water Management 71 (3):225-242. doi:10.1016/j.agwat.2004.09.003

Hart M, Ehret DL, Krumbein A, Leung C, Murch S, Turi C, Franken P (2014). Inoculation with arbuscular mycorrhizal fungi improves the nutritional value of tomatoes. Mycorrhiza 25 (5):359-376. doi:10.1007/s00572-014-0617-0

He Y, Yang R, Lei G, Li M, Li T, Zhan F, Li Y (2021). Arbuscular mycorrhizal fungus--induced decrease in phosphorus loss due to leaching in red soils under simulated heavy rainfall. Journal of soils and sediments 21 (2):881. doi:10.1007/s11368-020-02849-z

Herzog F, Prasuhn V, Spiess E, Richner W (2008). Environmental cross-compliance mitigates nitrogen and phosphorus pollution from Swiss agriculture. Environmental Science and Policy 11 (7):655-668. doi:10.1016/j.envsci.2008.06.003

Hetrick BAD (1989). Acquisition of phosphorus by VA mycorrhizal fungi and the growth responses of their host plants. In: Boddy L, Marchant R, Read DJ (eds) Nitrogen, Phosphorus and Sulphur Utilization by fungi Cambridge University Press, Cambridge, pp 205-226

Higo M, Isobe K, Yamaguchi M, Drijber RA, Jeske ES, Ishii R (2013). Diversity and vertical distribution of indigenous arbuscular mycorrhizal fungi under two soybean rotational systems. Biology and fertility of soils 49 (8):1085-1096. doi:10.1007/s00374-013-0807-5

Huang PM, Li Y, Sumner ME (2011). Handbook of soil sciences resource management and environmental impacts. Handbook of Soil Science, 2nd ed. edn. CRC Press, Boca Raton, Fla

Huang YZ, Zhong M, Wu W, Sui LH, Zhang C, Hao XW (2014). Effects of Arbuscular mycorrhizal fungi isolated from white clovers (*Trifolium repens* L) on soil bacteria and fungi. Chemistry in ecology 30 (2):118-132. doi:10.1080/02757540.2013.841892

Jakobsen I, Abbott LK, Robson AD (1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium subterraneum* L. 1. Spread of hyphae and phosphorus inflow into roots. The New Phytologist 120 (3):371-379. doi:10.1111/j.1469-8137.1992.tb01077.x

Jasper DA, Abbott LK, Robson AD (1989). Soil disturbance reduces the infectivity of external hyphae of vesicular- arbuscular mycorrhizal fungi. The New phytologist 112 (1):93-99. doi:10.1111/j.1469-8137.1989.tb00313.x

Javot H, Penmetsa RV, Terzaghi N, Cook DR, Harrison MJ (2007). A *medicago truncatula* phosphate transporter indispensable for the arbuscular mycorrhizal symbiosis. Proceedings of the National Academy of Sciences 104 (5):1720

Jennifer LP, David B, Roger TK, Andrew GS (2002). Mycorrhizal infection and high soil phosphorus improve vegetative growth and the female and male functions in tomato. The New phytologist 154 (1):255-264. doi:10.1046/j.1469-8137.2002.00366.x

Jiang XJ, Liu W, Chen C, Liu J, Yuan Z-Q, Jin B, Yu X (2018). Effects of three morphometric features of roots on soil water flow behavior in three sites in China. Geoderma 320:161-171. doi:10.1016/j.geoderma.2018.01.035

Kalbitz K, Solinger S, Park JH, Michalzik B, Matzner E (2000). Controls on the dynamics of dissolved organic matter in soils : A review. Soil science 165 (4):277-304. doi:10.1097/00010694-200004000-00001

Kang J, Amoozegar A, Hesterberg D, Osmond DL (2011). Phosphorus leaching in a sandy soil as affected by organic and inorganic fertilizer sources. Geoderma 161 (3):194-201. doi:10.1016/j.geoderma.2010.12.019

Khalvati MA, Hu Y, Mozafar A, Schmidhalter U (2005). Quantification of water uptake by arbuscular mycorrhizal hyphae and its significance for leaf growth, water relations, and gas exchange of barley subjected to drought stress. Plant biology (Stuttgart, Germany) 7 (6):706-712. doi:10.1055/s-2005-872893

Kim KY, Jordan D, McDonald GA (1997). Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biology and fertility of soils 26 (2):79-87. doi:10.1007/s003740050347

Kleinman PJA, Sharpley AN, Buda AR, McDowell RW, Allen AL (2011). Soil controls of phosphorus in runoff: Management barriers and opportunities. Canadian journal of soil science 91 (3):329-338. doi:10.4141/cjss09106

Köhl L, van der Heijden MGA (2016). Arbuscular mycorrhizal fungal species differ in their effect on nutrient leaching. Soil Biology and Biochemistry 94:191-199. doi:10.1016/j.soilbio.2015.11.019

Kohl L, van der Heijden MGA, Oehl F (2014). Agricultural practices indirectly influence plant productivity and ecosystem services through effects on soil biota.(Report). Ecological Applications 24 (7)

Kojima T, Hayatsu M, Saito M (1998). Intraradical hyphae phosphatase of the arbuscular mycorrhizal fungus, Gigaspora margarita. Biology and fertility of soils 26 (4):331-335. doi:10.1007/s003740050384

Kouno K, Tuchiya Y, Ando T (1995). Measurement of soil microbial biomass phosphorus by an anion exchange membrane method. Soil Biology and Biochemistry 27 (10):1353-1357. doi:10.1016/0038-0717(95)00057-L

Laird D, Fleming P, Wang B, Horton R, Karlen D (2010). Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma 158 (3):436-442. doi:https://doi.org/10.1016/j.geoderma.2010.05.012

Leff JW, Jones SE, Prober SM, Barberán A, Borer ET, Firn JL, Harpole WS, Hobbie SE, Hofmockel KS, Knops JMH, McCulley RL, La Pierre K, Risch AC, Seabloom EW, Schütz M, Steenbock C, Stevens CJ, Fierer N (2015). Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. Proceedings of the National Academy of Sciences - PNAS 112 (35):10967-10972. doi:10.1073/pnas.1508382112

Lehmann A, Veresoglou SD, Leifheit EF, Rillig MC (2014). Arbuscular mycorrhizal influence on zinc nutrition in crop plants - a meta-analysis. Soil Biology and Biochemistry 69:123-131. doi:10.1016/j.soilbio.2013.11.001

Lehmann J, Schroth G (2003). Nutrient leaching. In: Schroth G, Sinclair FL (eds) Tree, crops and soil Fertility. CAB International, Wallingford, UK, pp 151–166.

Lemarchand K, Masson L, Brousseau R (2004). Molecular biology and DNA microarray technology for microbial quality monitoring of water. Critical reviews in microbiology 30 (3):145-172. doi:10.1080/10408410490435142

Leung AK, Garg A, Coo JL, Ng CWW, Hau BCH (2015). Effects of the roots of *Cynodon dactylon* and *Schefflera heptaphylla* on water infiltration rate and soil hydraulic conductivity:effects of plant roots on infiltration characteristics and suction. Hydrological Processes 29 (15):3342-3354. doi:10.1002/hyp.10452

Li XL, George E, Marschner H (1991). Extension of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil. Plant and Soil 136 (1):41-48. doi:10.1007/BF02465218

Lindegren M, Persson P (2010). Competitive adsorption involving phosphate and benzenecarboxylic acids on goethite—Effects of molecular structures. Journal of colloid and interface science 343 (1):263-270. doi:10.1016/j.jcis.2009.11.040

Liu J, Aronsson H, Bergström L, Sharpley A (2012). Phosphorus leaching from loamy sand and clay loam topsoils after application of pig slurry. SpringerPlus 1 (1):1-10. doi:10.1186/2193-1801-1-53

Liu X-P, Bi Q-F, Qiu L-L, Li K-J, Yang X-R, Lin X-Y (2019). Increased risk of phosphorus and metal leaching from paddy soils after excessive manure application: Insights from a mesocosm study. The Science of the total environment 666:778-785. doi:10.1016/j.scitotenv.2019.02.072

M.H R, G.A C, D.C D (1994). Colonisation of wheat by VA-mycorrhizal fungi was found to be higher on a farm managed in an organic manner than on a conventional neighbour. Plant and soil 160 (1):33-40. doi:10.1007/BF00150343

Machado RMA, Oliveira MdRG (2005). Tomato root distribution, yield and fruit quality under different subsurface drip irrigation regimes and depths. Irrigation science 24 (1):15-24. doi:10.1007/s00271-005-0002-z

Marc IS (2015). The composition, leaching, and sorption behavior of some alternative sources of phosphorus for soils. Ambio 44 (S2):S207-S216. doi:10.1007/s13280-014-0615-7

Marschner H, Dell B (1994). Nutrient uptake in mycorrhizal symbiosis. Plant and Soil 159 (1):89-102. doi:10.1007/BF00000098

Marschner P, Crowley D, Lieberei R (2001). Arbuscular mycorrhizal infection changes the bacterial 16 S rDNA community composition in the rhizosphere of maize. Mycorrhiza 11 (6):297-302. doi:10.1007/s00572-001-0136-7

Martinez-Garcia LB, De Deyn GB, Pugnaire FI, Kothamasi D, van der Heijden MGA (2017) Symbiotic soil fungi enhance ecosystem resilience to climate change. Global change biology 23 (12):5228-5236. doi:10.1111/gcb.13785

Mathur N, Vyas A (1999). Improved biomass production, nutrient uptake and establishment of in vitro raised *Ziziphus mauritiana* by VA mycorrhiza. Journal of plant physiology 155 (1):129-132. doi:10.1016/S0176-1617(99)80153-9

McDowell RW, Worth W, Carrick S (2021). Evidence for the leaching of dissolved organic phosphorus to depth. The Science of the total environment 755 (Pt 1):142392-142392. doi:10.1016/j.scitotenv.2020.142392

McLaughlin M J FIRaTAR (1991). Operation of the phosphorus , sulphur and nitrogen cycles In: Barson RMGaMM (ed) Australia's renewable resources : sustainability and global change Bureau of rural resources, Canbera, Autralia, pp 67-116

Mello BL, Alessi AM, McQueen-Mason S, Bruce NC, Polikarpov I (2016). Nutrient availability shapes the microbial community structure in sugarcane bagasse compost-derived consortia. Scientific reports 6 (1):38781-38781. doi:10.1038/srep38781

Moreno SNJ, Docampo R (2013). Polyphosphate and its diverse functions in host cells and pathogens. PLoS pathogens 9 (5):e1003230-e1003230. doi:10.1371/journal.ppat.1003230

Murali A, Bhargava A, Wright ES (2018). IDTAXA: a novel approach for accurate taxonomic classification of microbiome sequences. Microbiome 6 (1):140-114. doi:10.1186/s40168-018-0521-5

Murphy J, Riley JP (1962). A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 27:31-36. doi:https://doi.org/10.1016/S0003-2670(00)88444-5

Nacoon S, Jogloy S, Riddech N, Mongkolthanaruk W, Kuyper TW, Boonlue S (2020). Interaction between phosphate solubilizing bacteria and arbuscular mycorrhizal fungi on growth promotion and tuber inulin content of *Helianthus tuberosus* L. Scientific reports 10 (1):4916-4916. doi:10.1038/s41598-020-61846-x

Nadejda AS, Marcel GAvdH, Johannes HCC, Mikhail IM, Vladimir GO, Mikhail NM, Asem AA, Peter MvB (2015). Quantitative assessment of the differential impacts of arbuscular and ectomycorrhiza on soil carbon cycling. The New phytologist 208 (1):280-293. doi:10.1111/nph.13447

Nelson NO, Parsons JE, Mikkelsen RL (2005). Field-scale evaluation of phosphorus leaching in acid sandy soils receiving swine waste. Journal of environmental quality 34 (6):2024-2035. doi:10.2134/jeq2004.0445

Neumann A, Torstensson G, Aronsson H (2012). Nitrogen and phosphorus leaching losses from potatoes with diiferent harvest times and following crops Field crops research 133:130-138

Nguyen TD, Cavagnaro TR, Watts-Williams SJ (2019). The effects of soil phosphorus and zinc availability on plant responses to mycorrhizal fungi: a physiological and molecular assessment. Scientific reports 9 (1):14880-14813. doi:10.1038/s41598-019-51369-5

Nkongolo KK, Narendrula-Kotha R (2020). Advances in monitoring soil microbial community dynamic and function. Journal of applied genetics 61 (2):249-263. doi:10.1007/s13353-020-00549-5

Nowaki H, Masatake M, Chiharu T, Ryo O, Mitsuru O, Tatsuhiro E (2010). Polyphosphate has a central role in the rapid and massive accumulation of phosphorus in extraradical mycelium of an arbuscular mycorrhizal fungus. The New phytologist 186 (2):285-289. doi:10.1111/j.1469-8137.2009.03168.x

Nuccio EE, Hodge A, Pett-Ridge J, Herman DJ, Weber PK, Firestone MK (2013). An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen

cycling during litter decomposition. Environmental microbiology 15 (6):1870-1881. doi:10.1111/1462-2920.12081

Nye PH, Tinker PB (1977). Solute movement in soil-root systems. Nature 272,564 (1977)

Pampolino MF, Urushiyama T, Hatano R (2000). Detection of nitrate leaching through bypass flow using pan lysimeter, suction cup, and resin capsule. Soil science and plant nutrition (Tokyo) 46 (3):703-711. doi:10.1080/00380768.2000.10409135

Panhwar QA, Ali A, Naher UA, Memon MY (2019). Chapter 2 - Fertilizer management strategies for enhancing nutrient use efficiency and sustainable wheat production. In: Chandran S, Unni MR, Thomas S (eds) Organic farming. Woodhead Publishing, pp 17-39. doi:https://doi.org/10.1016/B978-0-12-813272-2.00002-1

Parihar M, Meena V, Mishra P, Rakshit A, Choudhary M, Yadav R, Rana K, Bisht J (2019) Arbuscular mycorrhiza: a viable strategy for soil nutrient loss reduction. Archives of Microbiology 201 (6):723-735. doi:10.1007/s00203-019-01653-9

Paul EA (2014). Soil microbiology, ecology and biochemistry. Elsevier Science & Technology, Saint Louis

Pellegrino E, Öpik M, Bonari E, Ercoli L (2015). Responses of wheat to arbuscular mycorrhizal fungi: A meta-analysis of field studies from 1975 to 2013. Soil biology & biochemistry 84:210-217. doi:10.1016/j.soilbio.2015.02.020

Pote D, Daniel T, Delaune P (2009). Total phosphorus and dissolved phosphorus in water samples. In: Kovar J, Pierzynski J (eds) Methods of phosphorus analysis for soils, sediment,residuals and waters. Virginia tech University, Blacksburg, VA, USA, pp 113-117

Qian P, Schoenau JJ (2002). Practical applications of ion exchange resins in agricultural and environmental soil research. Canadian journal of soil science 82 (1):9-21. doi:10.4141/S00-091

Raghothama K, Karthikeyan A (2005). Phosphate acquisition. An International Journal on Plant-Soil Relationships 274 (1):37-49. doi:10.1007/s11104-004-2005-6

Ram AN, Asja B, Thomas A (2000). Analysis of phosphate acquisition efficiency in different Arabidopsis accessions. Plant physiology (Bethesda) 124 (4):1786-1799. doi:10.1104/pp.124.4.1786

Ramos-Zapata JA, Marrufo-Zapata D, Guadarrama P, Carrillo-Sánchez L, Hernández-Cuevas L, Caamal-Maldonado A (2012). Impact of weed control on arbuscular mycorrhizal fungi in a tropical agroecosystem: a long-term experiment. Mycorrhiza 22 (8):653-661. doi:10.1007/s00572-012-0443-1

Rasmussen N, Lloyd DC, Ratcliffe RG, Hansen PE, Jakobsen I (2000). ^{31}P NMR for the study of phosphorus metabolism and translocation in arbuscular mycorrhizal fungi.(Author abstract). Plant and Soil 226 (1):245

Réka N, David D, Nikolaus A, Iver J, Marcel B (2009). Mycorrhizal phosphate uptake pathway in tomato is phosphorus-repressible and transcriptionally regulated. The New phytologist 181 (4):950-959. doi:10.1111/j.1469-8137.2008.02721.x

Rillig M, Ramsey P, Gannon J, Mummey D, Gadkar V, Kapulnik Y (2008). Suitability of mycorrhiza-defective mutant/wildtype plant pairs (*Solanum lycopersicum* L. cv Micro-Tom) to address questions in mycorrhizal soil ecology. Plant and Soil 308 (1):267-275. doi:10.1007/s11104-008-9629-x

Rillig MC (2004). Arbuscular mycorrhizae and terrestrial ecosystem processes. vol 7. Oxford, UK. doi:10.1111/j.1461-0248.2004.00620.x

Rillig MC, Hoye AT, Carran A (2006a). Minimal direct contribution of arbuscular mycorrhizal fungi to DOC leaching in grassland through losses of glomalin-related soil protein. Soil Biology and Biochemistry 38 (9):2967-2970. doi:10.1016/j.soilbio.2006.03.022

Rillig MC, Mardatin NF, Leifheit EF, Antunes PM (2010). Mycelium of arbuscular mycorrhizal fungi increases soil water repellency and is sufficient to maintain water-stable soil aggregates. Soil biology & biochemistry 42 (7):1189-1191. doi:10.1016/j.soilbio.2010.03.027

Rillig MC, Mummey DL (2006). Mycorrhizas and soil structure. New Phytologist 171 (1):41-53. doi:10.1111/j.1469-8137.2006.01750.x

Rillig MC, Mummey DL, Ramsey PW, Klironomos JN, Gannon JE (2006b). Phylogeny of arbuscular mycorrhizal fungi predicts community composition of symbiosis-associated bacteria. FEMS microbiology ecology 57 (3):389-395. doi:10.1111/j.1574-6941.2006.00129.x

Roberts TL, Johnston AE (2015). Phosphorus use efficiency and management in agriculture. Resources, conservation and recycling 105:275-281. doi:10.1016/j.resconrec.2015.09.013

Roberts WM, George TS, Stutter MI, Louro A, Ali M, Haygarth PM (2020). Phosphorus leaching from riparian soils with differing management histories under three grass species. Journal of environmental quality 49 (1):74-84. doi:10.1002/jeq2.20037

Rouphael Y, Franken P, Schneider C, Schwarz D, Giovannetti M, Agnolucci M, Pascale SD, Bonini P, Colla G (2015). Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. Scientia horticulturae 196:91-108. doi:10.1016/j.scienta.2015.09.002

Schweiger PF, Thingstrup I, Jakobsen I (1999). Comparison of two test systems for measuring plant phosphorus uptake via arbuscular mycorrhizal fungi. Mycorrhiza 8 (4):207-213. doi:10.1007/s005720050236

Scott JT, Lambie SM, Stevenson BA, Schipper LA, Parfitt RL, McGill AC (2015). Carbon and nitrogen leaching under high and low phosphate fertility pasture with increasing nitrogen inputs. Agriculture, ecosystems & environment 202:139-147. doi:10.1016/j.agee.2014.12.021

Sharpley A, Jarvie HP, Buda A, May L, Spears B, Kleinman P (2013). Phosphorus legacy: overcoming the effects of past management practices to mitigate future water quality impairment. Journal of Environmental Quality 42 (5):1308-1326. doi:10.2134/jeq2013.03.0098

Sharpley AN, Rekolainen S (1997). Phosphorus in agriculture and its enviromental implications In: Tunney H, Carton OT, Brookes PC, Johnston AE (eds) Phosphorus loss from soil to water. CAB International, Wallingford, pp 1-53

Silva Elipe MV (2003). Advantages and disadvantages of nuclear magnetic resonance spectroscopy as a hyphenated technique. Analytica Chimica Acta 497 (1):1-25. doi:10.1016/j.aca.2003.08.048

Sims JT, Simard RR, Joern BC (1998). Phosphorus loss in agricultural drainage: Historical perspective and current research. Journal of Environmental Quality 27 (2):277-293

Smith ICP, Blandford DE (1995). Nuclear magnetic resonence spectrocospy. Analytical Chemistry 67 (12):509-518

Smith ME, Facelli JM, Cavagnaro TR (2018). Interactions between soil properties, soil microbes and plants in remnant-grassland and old-field areas: a reciprocal transplant approach. Plant and soil 433 (1-2):127-145. doi:10.1007/s11104-018-3823-2

Smith SE, Iver J, Mette G, Andrew S (2011). Roles of arbuscular mycorrhizas in plant phosphorus nutrition: interactions between pathways of phosphorus uptake in arbuscular mycorrhizal roots have important implications for understanding and manipulating plant phosphorus acquisition. Plant physiology (Bethesda) 156 (3):1050-1057. doi:10.1104/pp.111.174581

Smith SE, Read D (2008). Mycorrhizal symbiosis, third ed. Academic Press, Elsevier Ltd., Oxford

Smith SE, Read DJ, Read DJ (2008). Mycorrhizal Symbiosis. Elsevier Science & Technology, San Diego

Smith SE, Smith FA (2011). Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. Annual Review of Plant Biology, vol 62. doi:10.1146/annurev-arplant-042110-103846

Smith SE, Smith FA, Iver J (2004). Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal phosphorus uptake pathway is not correlated with mycorrhizal responses in growth or total phosphorus uptake. The New phytologist 162 (2):511-524. doi:10.1111/j.1469-8137.2004.01039.x

Solaiman MZ, Ezawa T, Kojima T, Saito M (1999). Polyphosphates in intraradical and extraradical hyphae of an arbuscular mycorrhizal fungus, *Gigaspora margarita*. Applied and Environmental Microbiology 65 (12):5604

Soudzilovskaia NA, van der Heijden MGA, Cornelissen JHC, Makarov MI, Onipchenko VG, Maslov MN, Akhmetzhanova AA (2015). Quantitative assessment of the differential impacts of arbuscular and ectomycorrhiza on soil carbon cycling. The New phytologist 208 (1):280-293

Stutter MI (2015). The composition, leaching, and sorption behavior of some alternative sources of phosphorus for soils. Ambio 44 (S2):S207-S216. doi:10.1007/s13280-014-0615-7

Taylor DL, Walters WA, Lennon NJ, Bochicchio J, Krohn A, Caporaso JG, Pennanen T (2016) Accurate estimation of fungal diversity and abundance through improved lineage-specific primers optimized for Illumina amplicon sequencing. Applied and environmental microbiology 82 (24):7217-7226. doi:10.1128/AEM.02576-1610.1128/AEM.02576-16.

Tinker PB (1978). Effects of vesicula-arbuscular mycorrhizas on plant nutrition and plant growth Physiologie Vegetable 16:743-75

Tran CTK, Watts-Williams SJ, Smernik RJ, Cavagnaro TR (2020). Effects of plant roots and arbuscular mycorrhizas on soil phosphorus leaching. Science of the Total Environment 722. doi:10.1016/j.scitotenv.2020.137847

Treseder KK (2013). The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. Plant and soil 371 (1/2):1-13. doi:10.1007/s11104-013-1681-5

Ulen B (1999). Leaching and balances of phosphorus and other nutrients in lysimeters after application of organic manures or fertilizers. Soil use and management 15:56-61

van der Heijden MGA (2010). Mycorrhizal fungi reduce nutrient loss from model grassland ecosystems. Ecology 91 (4):1163-1171. doi:10.1890/09-0336.1

van der Heijden MGA, Wiemken A, Sanders IR (2003). Different arbuscular mycorrhizal fungi alter coexistence and resource distribution between co-occurring plant. New Phytologist 157 (3):569-578. doi:10.1046/j.1469-8137.2003.00688.x

Vance CP (2001). Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. Plant Physiology 127 (2):390-397. doi:10.1104/pp.127.2.390

Verbruggen E, Kiers ET, Bakelaar PNC, Röling WFM, van der Heijden MGA (2012). Provision of contrasting ecosystem services by soil communities from different agricultural fields. Plant and Soil 350 (1-2):43-55. doi:10.1007/s11104-011-0828-5

Viereck N, Poul Erik H, Iver J (2004). Phosphate pool dynamics in the arbuscular mycorrhizal fungus *Glomus intraradices* studied by in vivo ^{31}P NMR spectroscopy. The New phytologist 162 (3):783-794. doi:10.1111/j.1469-8137.2004.01048.x

Vierheilig H, Coughlan AP, Wyss U, Piché Y (1998). Ink and vinegar, a simple staining technique for Arbuscular Mycorrhizal Fungi. Applied and Environmental Microbiology 64 (12):5004-5007

Wardle DA, Walker LR, Bardgett RD (2004). Ecosystem properties and forest decline in contrasting long-term chronosequences. Science 305 (5683):509-513. doi:10.1126/science.1098778

Watts-Williams SJ, Cavagnaro TR (2014). Nutrient interactions and arbuscular mycorrhizas: a meta-analysis of a mycorrhiza-defective mutant and wild-type tomato genotype pair. Plant and Soil 384 (1-2):79-92. doi:10.1007/s11104-014-2140-7

Watts-Williams SJ, Cavagnaro TR (2015). Using mycorrhiza-defective mutant genotypes of non-legume plant species to study the formation and functioning of arbuscular mycorrhiza: a review. Mycorrhiza 25 (8):587-597. doi:10.1007/s00572-015-0639-2

Watts-Williams SJ, Tyerman SD, Cavagnaro TR (2017). The dual benefit of arbuscular mycorrhizal fungi under soil zinc deficiency and toxicity: linking plant physiology and gene expression. Plant and Soil 420 (1-2):375-388. doi:10.1007/s11104-017-3409-4

Wheal MS, Fowles TO, Palmer LT (2011). A cost-effective acid digestion method using closed polypropylene tubes for inductively coupled plasma optical emission spectrometry (ICP-

OES) analysis of plant essential elements. Analytical Methods 3 (12):2854-2863. doi:10.1039/C1AY05430A

Wheeler RD, Baon JB, Alston AM, Smith SE (1992). Phosphorus efficiency of three cereals as related to indigenous mycorrhizal infection. Australian journal of agricultural research 43 (3):479. doi:10.1071/AR9920479

White PJ (2009). Efficiency of soil and fertilizer phosphorus use: reconciling changing concepts of soil phosphorus behaviour with agronomic information. Ex. Agric., vol 45. doi:10.1017/S0014479708007138

Wiedenfeld B, Enciso J (2008). Sugarcane Responses to Irrigation and Nitrogen in Semiarid South Texas. Agronomy journal 100 (3):665-671. doi:10.2134/agronj2007.0286

Wright ES (2016) Using Decipher v2.0 to analyze big biological sequence data in R. The R journal 8 (1):352. doi:10.32614/rj-2016-025

Xia H-Y, Zhao J-H, Sun J-H, Bao X-G, Christie P, Zhang F-S, Li L (2013). Dynamics of root length and distribution and shoot biomass of maize as affected by intercropping with different companion crops and phosphorus application rates. Field crops research 150:52-62. doi:10.1016/j.fcr.2013.05.027

Xu J, Liu S, Song S, Guo H, Tang J, Yong JWH, Ma Y, Chen X (2018). Arbuscular mycorrhizal fungi influence decomposition and the associated soil microbial community under different soil phosphorus availability. Soil biology & biochemistry 120:181-190. doi:10.1016/j.soilbio.2018.02.010

Zhang H, Liu T, Wang Y, Tang M (2019). Exogenous arbuscular mycorrhizal fungi increase soil organic carbon and change microbial community in poplar rhizosphere. Plant, soil and environment 65 (No. 3):152-158. doi:10.17221/2/2019-PSE

Zhang S, Yu X, Rillig M (2020). Arbuscular mycorrhiza contributes to the control of phosphorus loss in paddy fields. Plant and Soil 447 (1-2):623-636. doi:10.1007/s11104-019-04394-2

Ziane H, Meddad-Hamza A, Beddiar A, Gianinazzi S (2017). Effects of Arbuscular Mycorrhizal Fungi and Fertilization Levels on Industrial Tomato Growth and Production. International journal of agriculture and biology 19 (2):341-347. doi:10.17957/IJAB/15.0287

Zouari I, Salvioli A, Chialva M, Novero M, Miozzi L, Tenore G, Bagnaresi P, Bonfante P (2014) From root to fruit: RNA-Seq analysis shows that arbuscular mycorrhizal symbiosis may affect tomato fruit metabolism. BMC genomics 15 (1):221-221. doi:10.1186/1471-2164-15-221