Carbon and phosphorus trading in the arbuscular mycorrhizal symbiosis

Von der Naturwissenschaftlichen Fakultät der
Gottfried Wilhelm Leibniz Universität Hannover

zur Erlangung des Grades
Doktor der Naturwissenschaften (Dr. rer. nat.)

genehmigte Dissertation
von
Alberto Andrino de la Fuente, Máster (Spain)

2019

Referent: Prof. Dr. Georg Guggenberger

Korreferentin: Prof. Dr. Traud Winkelmann

Tag der Promotion: 07.05.2019

Para Inma, Gabriel y Leo

Abstract

Phosphorus (P) is a limiting element in natural and managed ecosystems, with most organic and inorganic P being associated with secondary minerals in acidic soils, and in the form of sparingly soluble calcium phosphates in calcareous ones. For enhancing P availability of these sources, most terrestrial plants establish symbiotic association with arbuscular mycorrhiza fungi (AMF), which in return constitute a major sink of plant photosynthates. While studies on plant P nutrition supplied by soluble inorganic P abound, there is a knowledge gap on the AMF's ability to mobilise P from primary minerals such as apatite (AP) or orthophosphate (OP) and phytic acid (PA) bound to iron secondary minerals (E.g. goethite -GOE-) in highly weathered soils. Soil P forms are likely differing in their plant acquisition costs, with insoluble organic (E.g. GOE-PA) and inorganic forms (E.g. GOE-OP and AP) are the metabolically most expensive. For AM symbioses it has not yet been shown whether C allocation into the AMF may increase when less accessible soil P forms are available, given that they are available to the AMF at all.

I addressed three objectives in the current dissertation. The first objective was to clarify whether an AM plant can take up P from less accessible sources exclusively through the mycorrhizal pathway. The second objective was to explore the carbon (C)–P trading between arbuscular mycorrhizal (AM) plants, along with the ability of this symbiosis to incorporate P derived from differently accessible P sources. Finally, to elucidate the mechanisms involved in the mobilisation of the different P sources by the AM symbiosis was the third objective. For the first and second objectives, I hypothesized (1) AM plants accessing the different P sources will mobilise P in different amounts and rates and (2) the less accessible P sources will require larger photoassimilate investments into the AMF, resulting in differing trading costs per P unit compared to soluble forms. For the third objective I hypothesized (1) P mobilisation will enhance the photosynthetic function in case the AM plant has access to a P source in order to maintain an optimal photosynthetic transfer to the AMF. (2) the AMF development correlates positively with P incorporation rates into the plant tissues, no matter which P source was mobilised; (3) the P mobilisation from the different sources will change the low molecular weight organic acid (LMWOA) profile, with more abundant di/tricarboxylic LMWOA in case of the less accessible P sources.

I designed a mesocosms made of a plant compartment containing *Solanum lycopersicum* plants mycorrhized with the AMF *Rhizophagus irregularis* DAOM 197198, and a fungal compartment where exclusively the AMF could access and mobilise the different P sources.

ABSTRACT

The C-P trading between an AM plant and its ability to incorporate P derived from differently accessible P sources was tested on fungal compartments containing OP, PA, AP, GOE-PA and GOE-OP, while the identification of mechanisms was checked for OP, PA, GOE-OP and GOE-PA. Over different time course experiments, I measured plant P stocks and the C budget to access the P sources was estimated by measuring the respired CO_2, the organic C, the phospholipid fatty acid (PLFA) 16:1ω5c (mycelium biomass) and the neutral lipid fatty acid (NLFA) 16:1ω5c (mycelial energy storage) at the fungal compartment. In order to determine the mechanisms, photosynthetic capacity and mycorrhization rates were measured at the plant compartment, while LMWOA and PLFA microbial biomarkers were monitored at the fungal compartment.

All AM plants incorporated P derived from all five sources through the mycorrhizal pathway. They did this at different rates and differing photosynthetic investment costs per unit of P incorporated, yielding differing C:P trading rates. Arbuscular mycorrhizal plants mobilising PA, AP, GOE-OP and GOE-PA caused a larger mycelium infrastructure (PLFA) and a higher energy storage (NLFA) as compared to the OP. All AM plants with access to a P source preferentially stored P in their shoots. This drove up photosynthetic capacity and was accompanied by an improved photosynthetic P use efficiency. Phosphorus incorporation in AM plants correlated with abundance of AMF and bacterial biomarkers in case of PA and GOE-PA, but not in case of GOE-OP. I found di/tricarboxylic LMWOA in treatments containing PA, GOE-PA and GOE-OP before and during the P incorporation, pointing to a ligand exchange mechanism to mobilise the P.

The different C investments into P allocation from differently accessible sources suggests a broad nexus between P mining strategies, resource partitioning in soil, and the amounts of C accumulated in terrestrial soils. Furthermore, the current dissertation provides the first evidence on the ability of an AM plant to incorporate P derived from an organic source bound to a secondary mineral (GOE-PA), opening the possibility to access the set of different P forms in soils, and thus reduce the application of fertilizers based on phosphate rock.

Keywords: Carbon-Phosphorus trading, resource partitioning, organic phosphorus, inorganic phosphorus, primary mineral, secondary mineral, arbuscular mycorrhiza, PLFA 16:1ω5c, NLFA 16:1ω5c, goethite, apatite, phytate, photosynthesis, LMWOA.

Zusammenfassung

Phosphor (P) ist sowohl in natürlichen und bewirtschafteten Ökosystemen ein limitierendes Element. Organischer und anorganischer Phosphor assoziiert in sauren Böden häufig mit sekundären Mineralien, während er in kalkhaltigen Böden oft als schwerlösliches Kalziumphosphaten vorkommt. Um die Verfügbarkeit dieser P- Quellen zu verbessern, gehen die meisten Landpflanzen eine symbiotische Assoziation mit arbuskulären Mykorrhizapilzen (AMF) ein, welche von der Pflanze mit Photoassimilaten alimentiert wird, und somit eine nicht zu vernachlässigende Senke für Kohlenstoff darstellt. Während Studien über die pflanzliche P-Versorgung durch lösliches, anorganisches P zahlreich vorhanden sind, gibt es eine Wissenslücke über die Fähigkeit der AMF, Phosphor aus primären Mineralien wie Apatit (AP) oder Orthophosphat (OP) und Phytinsäure (PA) zu mobilisieren, die an sekundäre Eisenminerale in stark verwitterten Böden gebunden sind (z.B. Goethit -GOE-). Die Mobilisierung dieser unterschiedlichen P-Formen erfordert dabei unterschiedlich hohe Energieinvestitionen seitens der Pflanze. Unlösliche organo-mineralische Komplexe (z.B. GOE-PA), wie auch anorganische Assoziate (z.B. GOE-OP) oder Primärminerale (AP) stellen diesbezüglich vermutlich die höchsten metabolischen Anforderungen. Für die AM-Symbiose ist es allerdings bisher noch nicht nachgewiesen, ob die Erschließung weniger zugänglicher P-Formen im Boden tatsächlich höhere Photoassimilatinvestitionen nach sich ziehen, bzw. ob diese überhaupt für die AM zugänglich sind.

In meiner Dissertation habe ich drei Ziele verfolgt. Mein erstes Ziel war es, zu klären, ob eine AM überhaupt P aus weniger zugänglichen Quellen mobilisieren kann, wenn ausschließlich der Aufnahmepfad über die Mykorrhiza selbst zur Verfügung steht. Das zweite Ziel war die Erforschung des Kohlenstoff-(C)-P-Handels zwischen den Symbiosepartnern in Abhängigkeit der unterschiedlichen Verfügbarkeit der P-Quellen. Schließlich war es das dritte Ziel, die Mechanismen der Mobilisierung der verschiedenen P-Quellen durch die AM-Symbiose aufzuklären. Für das erste und zweite Ziel ergaben sich folgende Hypothesen: (1) AM-Pflanzen, die auf die verschiedenen P-Quellen zugreifen, mobilisieren P in abweichenden Raten und Mengen, die sich wiederum aus der Beschaffenheit der P Quellen ergeben. (2) Weniger zugängliche P-Quellen erfordern dabei größere Investitionen an Photoassimilaten in die AMF, was zu höheren Handelskosten pro P-Einheit im Vergleich zu leichter zugänglichen P-Quellen führt.

Für das dritte Ziel wurden folgende Hypothesen überprüft: (1) Eine erhöhte P-Mobilisierung seitens der AM erhöht auch die Photosyntheserate. (2) Die AMF-Entwicklung korreliert positiv mit den P-Inkorporationsraten in das pflanzliche Gewebe, unabhängig davon, welche P-Quelle mobilisiert wurde; (3) die P-Mobilisation aus den verschiedenen Quellen verändert die Signatur der niedermolekularen organischen Säuren (LMWOA) im System, und zwar hin zu einem höheren Anteil an Di- und Tricarbonsäuren im Falle der weniger zugänglichen P-Quellen.

Ich verwendete hierzu Mesokosmen, die aus einem Pflanzenkompartiment, welches mit durch *Rhizophagus irregularis* DAOM 197198 mykorrhizierten *Solanum lycopersicum* Pflanzen bestockt wurden, sowie einem reinem Pilzkompartiment, in dem ausschließlich die AMF auf die verschiedenen P-Quellen zugreifen und diese mobilisieren konnte, bestanden. Im Pilzkompartiment wurde der C-P-Handel über die AM in Abhängigkeit unterschiedlich zugänglicher P-Quellen (OP, PA, AP, GOE-PA und GOE-OP) untersucht. Die Prozessaufklärung der Mobilisierung wurde hier ebenfalls für OP, PA, GOE-OP und GOE-PA durchgeführt. Der Phosphorgehalt in der Pflanze wurde ebenso wie die assimilierte Menge an CO_2, der Gehalt an organischem Bodenkohlenstoff, sowie das Verhältnis zwischen der Phospholipidfettsäure (PLFA) 16:1ω5c (Biomasse des Myzels) und der neutralen Lipidfettsäure (NLFA) 16:1ω5c (Energiespeicherfunktion im Myzel) gemessen. Um die Mechanismen zu bestimmen, wurden photosynthetische Raten und Mykorrhizationsraten im Pflanzenkompartment gemessen, während LMWOA und PLFA als mikrobielle Biomarker im Pilzkompartiment bestimmt wurden.

AM-Pflanzen konnten im Laufe des Experimentes P aus allen angebotenen P-Quellen inkorporieren, wobei P zwingend und stets durch die AMF mobilisiert wurde. Diese Aufnahme in die Pflanze erfolgte je nach vorliegender P-Quelle in unterschiedlichen Raten und zu unterschiedlichen photosynthetischen Investitionskosten pro P-Einheit, was in der Summe zu unterschiedlichen C:P-Handelsraten führte. Arbuskuläre Mykorizapflanzen, die PA, AP, GOE-OP und GOE-PA über ihren Pilzpartner mobilisierten, unterhielten eine größere Myzelinfrastruktur (PLFA) im Vergleich zu solchen, die OP als P-Quelle zur Verfügung hatten. Dies ging mit erhöhten Energierücklagen im Myzel (NLFA) einher. Alle AM-Pflanzen mit Zugang zu einer P-Quelle reicherten P in ihrer Sprossachse an, was zu einer höheren Photosyntheserate und einer verbesserten photosynthetischen P-Nutzungseffizienz führte. Die Höhe der Phosphoraufnahme durch AM-Pflanzen mit Zugang zu PA und GOE-PA korrelierte mit der Abundanz von AMF und bakteriellen Biomarkern. Während der P- Aufnahme aus PA, GOE-PA und GOE-OP erhöhte sich der Anteil an Di und Tricarbonsäuren unter den LMWOA, was auf einen Ligandenaustausch als Mobilisationsmechanismus hindeutet.

Die unterschiedlichen C-Investitionen in die P-Allokation aus unterschiedlich zugänglichen Quellen deuten auf einen breiten Zusammenhang zwischen P-Mining-Strategien, Ressourcenallokation im Boden und den dabei akkumulierten C-Mengen hin. Darüber hinaus stellen meine Ergebnisse den ersten Beweis für die Fähigkeit einer AM-Pflanze dar, P aus einer organischen Quelle aufzunehmen, die an ein sekundäres Mineral gebunden ist (GOE-PA). Die Möglichkeit verschiedene und vermeintlich unzugängliche P-Quellen im Boden mittels Mykorrhiza für Pflanzen verfügbar zu machen, eröffnet eine potentiell interessante und ressourcenschonendere Alternative zur herkömmlichen Phosphordüngung.

Acknowledgements

Firstly, I would like to express my sincere gratitude to Dr. Jens Boy and Prof. Dr. Georg Guggenberger for their continuous support of my Ph.D study and related research, for their patience, motivation, and immense knowledge. Their guidance helped me in all the time of research and writing of this thesis. I could not have imagined having better advisors and mentors for my Ph.D study. Thank you.

Besides my advisors, I would like to thank the professors belonging to the project “Signaling at the Plant-Soil Interface”, Prof. Dr. Traud Winkelmann, Prof. Manfred K. Schenk, Prof. Dr. Helge Küster and Prof. Dr. Marcus Andreas Horn for their support in technical and logistical aspects that I required during the thesis. I would also like to thank the support received by Prof. Dr. Robert Mikutta within the framework of the DFG priority program SPP 1685 “Forest Strategies for limited Phosphorus Resources”.

My research would have been impossible without the aid and support of Dr. Leopold Sauheitl. Thank you Poldi!

My sincere thanks also goes to the Soil Science Lab Team: Silke Bokeloh, Elke Eichmann-Prusch, Ulrike Pieper, Waldemar Walter, Pieter Wiese, Heike Steffen, Michael Klatt and Anne Herwig. Without their precious support it would not be possible to conduct this research.

I thank my colleagues Norman Gentsch, Norbert, Bischoff, Fabian Kalks, Susanne K. Woche, Stefan Dultz, Aline Figueiredo, Markus Koch and Patrick Liebmann for their stimulating discussions and support in the last five years.

Last but not the least, I would like to thank my parents and my two brothers for supporting me in the distance, throughout writing this thesis and in my life in general. I send you a big hug, Grandma, Grandpa and Mario, wherever you are.

This thesis is dedicated to the three most important people in my life, my two sons Gabriel and Leo, and to you Inma, if we have finished a thesis taking care of our two precious children, then there is no difficult path when we are together.

Table of Contents

Abbreviations

Al	aluminum
AMF	arbuscular mycorrhizal fungi
AP	apatite
C	carbon
Ca	calcium
Fe	iron
CO_2	carbon dioxide
GC	gas chromatography
FAME	fatty acid methyl ester(s)
GOE	goethite
GOE-OP	orthophosphate bound to goethite
GOE-PA	phytic acid bound to goethite
H_2O	water
ICP-MS	inductively coupled plasma mass spectrometry
IRMS	isotope ratio mass spectrometry
LMWOA	low molecular weight organic acid(s)
N	nitrogen
NLFA	neutral lipid fatty acid(s)
O	oxygen
OC	organic carbon
OP	orthophosphate
P	phosphorus
PA	phytic acid
PCA	principal components analysis
PLFA	phospholipid fatty acid(s)

1. State of The Art and Hypothesis

1.1 Thesis Structure

The following dissertation has been structured in five chapters. The first chapter deals with the most relevant bibliography on plant phosphorus nutrition through the arbuscular mycorrhizal symbiosis, and proposes a series of questions to be investigated, to finally formulate the working hypothesis. The following three chapters belong to three separate studies formatted as journal papers. The Study 2 has been already submitted to the journal *Frontiers in Environmental Science*, special research topic *Rhizosphere Functioning and Structural Development as Complex Interplay between Plants, Microorganisms and Soil Minerals* and it is undergoing the peer review process. While the other two studies will soon be submitted. The last chapter recaps and discusses the main discoveries of the current study.

1.2 Phosphorus in the Soil-Plant System

1.2.1 Phosphorus: a Finite Resource

Phosphorus (P) is an indispensable, irreplaceable and finite element for all life forms in the Earth's ecosystem (Tiessen et al., 2011). During the last 150 years, approximately 1,000 million tons of easily mineable deposits of phosphate rock have been added to the environment as soluble phosphate fertilizers. Estimated P losses are of the same order of magnitude as the annual world consumption of fertilizer-P, since most of the P losses negatively enhance the biological activity in fresh water bodies (eutrophication) together with the loss by burial of P in sediments of coastal ocean waters (Rowe et al., 2016; Tiessen et al., 2011). In the middle of the previous century the First Green Revolution, mostly based on the use of high-yielding varieties of cereals in association with chemical fertilizers and agro-chemicals, allowed food production to keep pace with worldwide population growth (Herder et al., 2010). But nowadays increasing crop yield through a growing use of rock-based phosphate fertilizers and water has reached a maximum (Herder et al., 2010). Rock phosphate deposits are a finite resource and could become scarce within the current century, making P fertilizers more expensive (Gilbert, 2009) and one of the solutions to the problem lie in developing ways to use it more efficiently (Elser and Bennett, 2011). Because of that, all plant strategies that increase P uptake and use efficiencies will be increasingly valuable in the upcoming future (Smith and Smith, 2011) and this knowledge will eventually contribute to a more efficient P usage in agroecosystems (Bünemann et al., 2011). Thus, the foundations of a Second Green Revolution must be built on a systemic-holistic approach based on the use of the ecosystem services, in order to achieve sustainable yield and crop quality together with high energy efficiency and low environmental impact (Pellegrino and Bedini, 2014). In this context, the ecosystem services provided by soil biota to maintain soil quality, plant health and soil resilience are extremely relevant (Smith and Read, 2008). Particularly, soil microbes that form symbiosis with plant roots have become more important, as they offer a biological alternative to promote plant growth and reduce inputs of phosphate fertilizers (Gianinazzi et al., 2010).

1.2.2 Understanding Soil Phosphorus

Unlike carbon (C), oxygen (O) or nitrogen (N) cycles, the global P cycle is a sedimentary cycle, which originates from P primary mineral deposits and sediments from crust rocks (Arif et al., 2017). Over time, geochemical and biological processes have been regulating the P availability, transforming inorganic phosphorus into stable forms of organic and inorganic phosphorus in soil. Geochemical processes, at the global scale and over the long term, link the movement and distribution of P between the terrestrial soil and the oceanic sediment pools. In the short-term, biological processes influence P distribution since the majority of the plant-available P is derived from soil organic matter (Cross and Schlesinger, 1995).

Walker and Syers (1976) proposed the proportion of various P forms may change as the soil develops, suggesting that the proportion of P in labile, non-labile, non-occluded, and occluded fractions should vary along a weathering intensity gradient. Ecosystems of young and slightly weathered soils P should be found in primary minerals (E.g. apatite -AP-); in those ones with a moderate weathering level, most P will be found in organic compounds or adsorbed to secondary clay minerals (E.g. goethite -GOE-). While in deeply weathered soil ecosystems, P will be in the non-labile, occluded, or stable organic forms. P is the least mobile nutrient in most soils (Devau et al., 2009), in acidic soils, a major fraction of P is adsorbed to iron (Fe) and aluminium (Al) (hydr)oxides, while it is found in the form of sparingly soluble calcium (Ca) phosphates in calcareous soils (Li et al., 2018). P can be found in organic and inorganic forms, being the organic P a major component in soils, with phytic acid (PA) representing >50% of the organic P, in which the pH is low and is present in compounds that must be solubilized and mineralized prior to their incorporation by plants (Turner, 2008). While inorganic P is in form of sparingly soluble P minerals such as apatites (AP), representing up to 75–90%, especially in the more calcareous soil types (Pel et al., 2018), and bound to Fe and Al compounds in acidic soils (Li et al., 2018).

1.2.3 Plant Phosphorus Nutrition

P is an essential macronutrient for plant growth and productivity (Rowe et al., 2016), making up about 0.2% of a plant's dry weight. It is an essential element of key molecules such as phospholipids, nucleic acids (Schachtman et al., 1998). It is involved in the accumulation and release of energy during cellular metabolism (E.g. ATP), and is also involved in controlling key enzyme reactions and in the regulation of metabolic pathways (Mishra et al., 2014).

In natural ecosystems, plants have evolved to respond to P starvation by increasing the ability of their root systems to acquire P from the soil (George et al., 2011), as 95–99% of soil P occurs in forms that are not directly available to plants (Javaid, 2009; Osorio and Habte, 2001). In order to be available to plants, OP must be either desorbed or solubilised, and organic P must be mineralised to release the OP (Cross and Schlesinger, 1995; George et al., 2011). Plants exclusively take up P in inorganic form either as primary OP ($H_2PO_4^-$) or secondary OP (HPO_4^{2-}) from soil solution (Becquer et al., 2014), but soil P levels available to plants are limiting for growth, since the soil concentration ranges from 1 to 10μM and this is about 1000-fold lower than plant cellular concentrations (Smith and Smith, 2012).

Plant soil P availability needs to be systemically evaluated because it is highly associated with P dynamics and transformation among the various P pools (Shen et al., 2011). Soil P pools are in equilibrium with OP forms ($H_2PO_4^-$, HPO_4^{2-}) in the soil solution and govern soil P cycle by the processes of desorption-sorption, in the case of adsorbed P (E.g. GOE-OP, GOE-PA); dissolution-precipitation, in the case of mineral P (E.g. AP); and mineralization-immobilisation, in the case of organic P (E.g. PA) (Arif et al., 2017).

1.2.3.1 Plant Strategies to Overcome Phosphorus Deficiency

To overcome the soil P limitation plants developed different strategies to acquire P from soil solution (George et al., 2011; Giles et al., 2012; Richardson et al., 2009):

- Increasing the root-soil interface and enabling a greater soil volume exploration and the exploitation of localized patches of high OP availability (E.g. Cluster roots).
- Solubilizing P trapped in complexes by roots secretions such as low molecular weight organic acids (LMWOA) and phosphatases (E.g. Acid phosphatase, phytase) to mobilise the OP from inorganic and organic P sources.
- Forming symbiotic associations with mycorrhizal fungi and/or P solubilizing and/or mineralizing microorganisms. Mycorrhizal symbioses are associations of plant roots or rhizoids with fungi that, at least under some conditions, are beneficial to both partners (E.g. Arbuscular symbiosis).

- Regulating plants own metabolism by the expression and regulation of transporters and the remobilisation of plant P pools.

1.2.4 Phosphorus Nutrition Mediated by the Arbuscular Mycorrhizal Symbiosis

1.2.4.1 Arbuscular Mycorrhizal Symbiosis

The arbuscular mycorrhizal fungi (AMF) establish a species-unspecific association with almost 80% of earth plant species (Ryan et al., 2012; Smith and Read, 2008). The AMF are symbiotic cosmopolitan soil microbes and a functionally critical component of terrestrial ecosystems (Stürmer et al., 2018). AMF function is strongly influenced by the soil environment, particularly those factors that control mineral fertility and generally, AM benefits are greater in P-poor soils than in P-rich ones (Koide, 1991). AMF also have shown the ability to accelerate organic matter decomposition and uptake significant contents of nitrogen (Jansa et al., 2011).

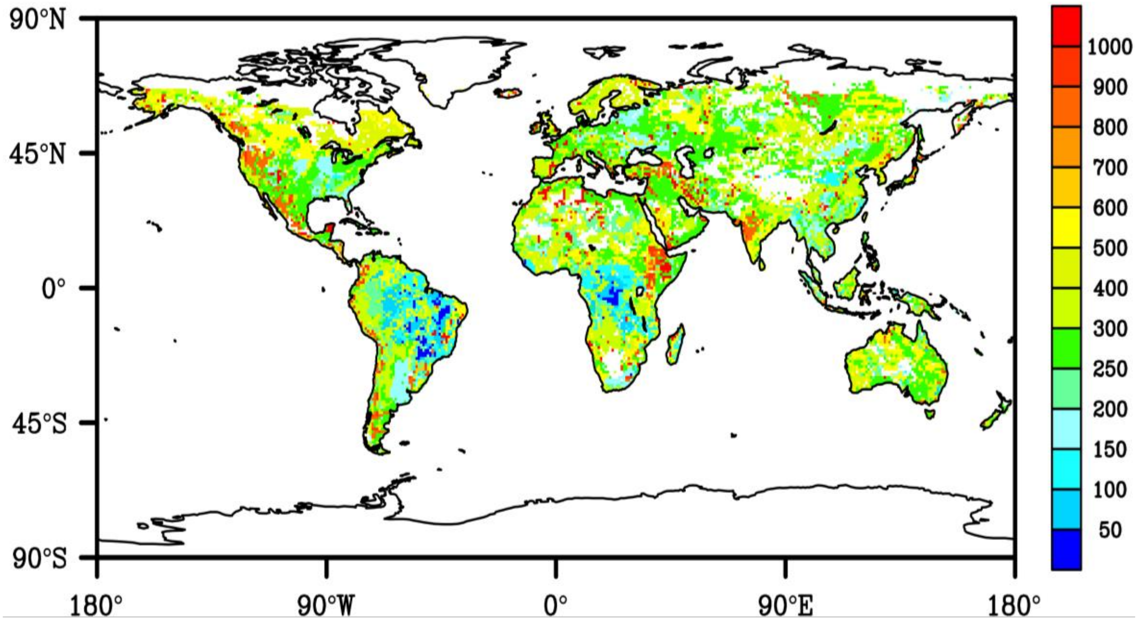


Figure 1.1. Global distribution of the estimated Total P (g P m^2). Taken from Yang et al.(2013).

Most of the plant establishing an AM symbiosis develop in the understory, in grasslands and in tropical forests (Jansa et al., 2011), where P is lowest (Figure 1.2) and soils have gone through millions of years of soil development and have lost most of their original P through leaching or erosion (Yang et al., 2013). AMF plays a crucial role in the P acquisition of plants, enhancing the soil volume that can be explored (Lambers et al., 2015), since the mycorrhizal hyphae are capable of penetrating smaller soil pores (5–30 mm) than the roots (>50–100 mm), thus expanding access to the soil (Jansa et al., 2011). By increasing soil exploration, AM plants are able to acquire resources far beyond the root depletion zone, unlike root hairs, which only extend a few mm (Jansa et al., 2011). AMF's hyphae also represents the lowest construction and maintenance costs (2–20µm diameter), compared to root hair development (100–500µm diameter) (Hodge, 2016; Leake and Read, 2016). This symbiotic association has been selected over the last millions of years, since the least costly modes of P acquisition are supposed to be favoured by natural selection and during the assembly of plant communities by competition (Raven et al., 2018). Arbuscular mycorrhizal (AM) symbiosis was established in the early Devonian, 400 million years ago, at the dawn of terrestrial plant evolution, between the ancestral vascular plants and AMF belonging to the phylum Glomeromycota (Figure 1.3) (Jansa et al., 2011).

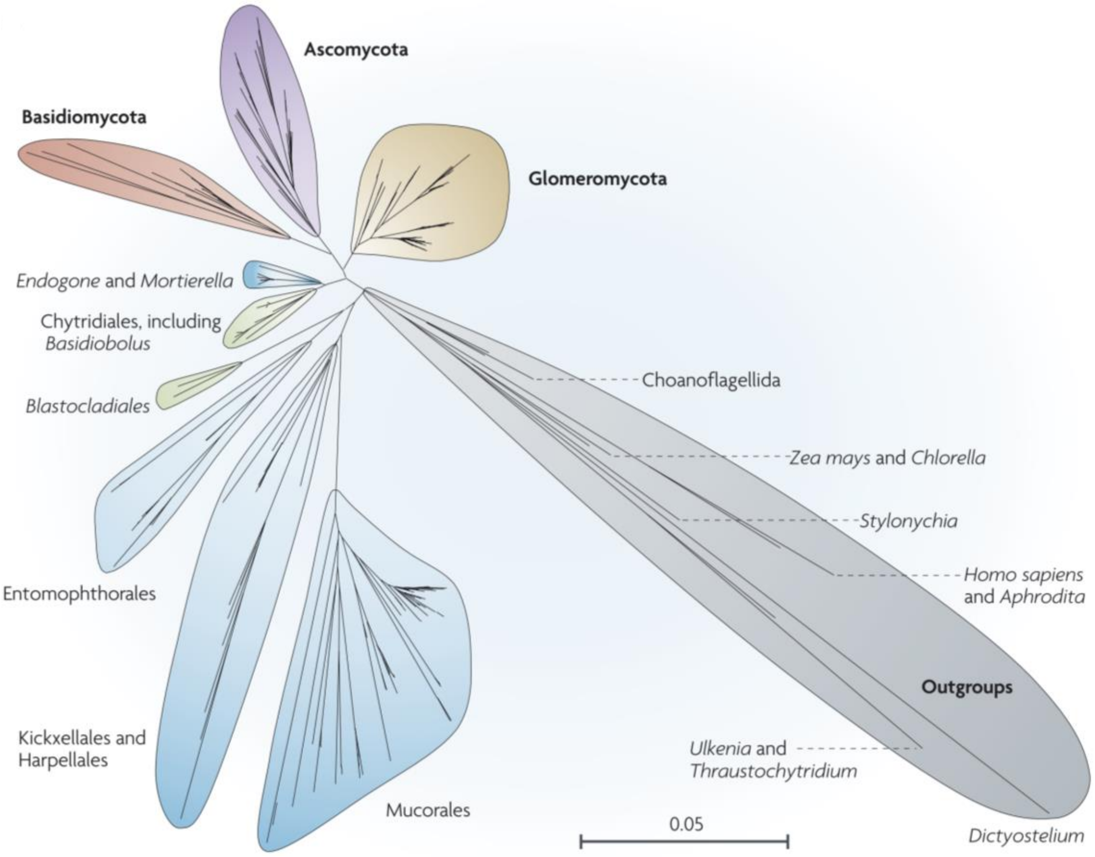


Figure 1.2. A phylogenetic tree showing the phylum Glomeromycota in relation to other fungal lineages. Taken from Parniske (2008).

1.2.4.2 Phosphorus uptake

The AM fungi have shaped the morphology and physiology of plant roots becoming obligatory biotrophs as their ecological and physiological requirements became increasingly interlaced with plant roots functions (Johnson, 2010). AMF colonizes the root by means of an attachment structure called hyphopodia on the root surface of the host plant. Then the hyphae passes through the outer cell layers until it reaches the internal cortical cells, where they form the arbuscules (from the Latin 'arbusculum', meaning bush or little tree), which are highly branched (Figure 1.4). The arbuscules are surrounded by the plant periarbuscular membrane (PAM) which, together with the arbuscular membrane, constitutes the main symbiotic interface for the exchange of nutrients between the fungus and the plant. The apoplastic interface between the fungal plasma membrane and the plant-derived PAM is called the periarbuscular space (PAS) (Keymer and Gutjahr, 2018; Parniske, 2008).

Uptake of P from the soil solution to the fungal hyphae cells is mediated by specific fungal P transporters (Boldt et al., 2011). The P acquired by the AMF fungi has two different destinations, the first one is to satisfy the physiological demand of the fungus, and the rest is transported to the plants or stored in the hyphae. For storage or long-distance transport of P through the hyphae, P becomes part of larger molecules called polyphosphates (Solaiman et al., 1999). Polyphosphates are transported along the hyphae and after a process of depolymerisation, the shorter chains of polyphosphates seem to be the immediate source of P for the plants that are exchanged at the arbuscular level (Jansa et al., 2011).

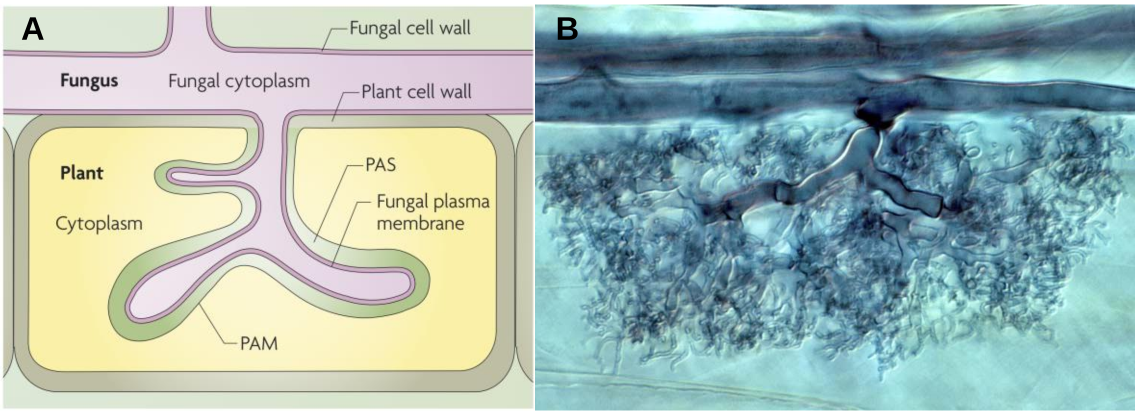


Figure 1.3. A. A sketch of an arbuscule, the symbiotic structure and arbuscular mycorrhiza. PAM, plant periarbuscular membrane; PAS, periarbuscular space. Taken from Parniske (2008). B. Arbuscule of *Glomus mosseae* in root cortex cells. Taken from (Brundrett et al., 1996)

1.2.4.3 Carbon Exchange

AMF are heterotrophic organisms and in exchange for the P acquisition, stimulate the host photosynthetic activity and AM plants allocate 4-20% of the recently C fixed into the AMF structures (Tomè et al., 2016). The C translocation into the fungi represents a major sink of plant photosynthates (Boldt et al., 2011; Nottingham et al., 2010), which are derived into fungal growth, respiration, and biological maintenance (Jansa et al., 2011). It has long been assumed that AMF use sugars from the plant as precursors for lipid biosynthesis as the major sources of organic C. However, *de novo* biosynthesis of fungal fatty acids was only observed inside colonized roots and not in extraradical mycelia or spores (Keymer et al., 2017). Recently, several publications have exhibited that the AMF model organism *Rhizophagus irregularis* cannot synthesize fatty acids *de novo* from plant's glucose and glycerol (Bravo et al., 2017; Jiang et al., 2017; Keymer et al., 2017; Luginbuehl et al., 2017). The AM fungal genome of *R. irregularis* lacks genes encoding type I multidomain fatty acid synthases, which synthesize palmitic acid (C16:0) in fungi, and can only elongate them, requiring the host plant to produce them (Keymer et al., 2017). The studies propose a model in which the sources of C for mutualistic AMF include fatty acids exported from the host plant, as well as sugars, providing the fungi with a robust source of C for their metabolic needs and making the AMF an obligate biotroph (Figure 1.5).

The AMF store C mainly in the form of lipids (Bago et al., 2002; Trépanier et al., 2005), and the predominant storage form is triacylglycerol and the major proportion of fatty acids found in AMF is composed of 16:0 (palmitic acid), and of 16:1w5 (palmitvaccenic acid) (Keymer et al., 2017). The AMF specific 16:1w5 fatty acid are stored in the form of glycerolipids (neutral lipids) but also incorporated into membrane phospholipids (Olsson and Johansen, 2000). The C stored at the AMF are important sources and components of the soil organic C pool, contributing to both fast and slow pools (Zhu and Miller, 2003). The AMF grows from colonized roots into the soil and form an extraradical mycelium consisting of recalcitrant runner hyphae, short-lived absorbing hyphae and persistent reproductive spores. Staddon et al. (2003) estimated a turnover rate for the extraradical mycelium of 5-6 days, but it is unlikely that it can be generalized, because the AM fungal hyphae are made of chitin and they produce glomalin, two recalcitrant substances which are known to have a long residence time in the soil (Olsson and Johnson, 2005). The deposition of slow cycling organic compounds such as chitin and glomalin contribute to soil structure by the creation a skeletal structure to hold soil particles together and promoting the formation micro and macro-aggregates, which protect organic matter from microbial attack (Cardoso and Kuyper, 2006; Cheng et al., 2012; Willis et al., 2013) and contribute to the maintenance of an efficient and functional soil ecosystem in both agricultural and forestry environments (Dalpé et al., 2012; Graham et al., 1995). The presence of AMF significantly modifies soil conditions in the rooting zone (aggregation, wettability and biological activity), modulating intra- and interspecific competition within the plant community, and affecting soil microbial communities. In this regard, an increasing body of literature states that after the plant photoassimilates reach the fungal symbiont, AMF may act as a hub for translocating fresh plant-derived C to soil to the hyphospheric microbes on a rapid timescale. The publications suggest a synergistic cooperation may occur, that could affect the diversity, structure and the strength of AMF associated microbial communities (Agnolucci et al., 2015; Choudhary et al., 2017; Gui et al., 2017; Kaiser et al., 2015; Krishnamoorthy et al., 2016; Nuccio et al., 2013; Paterson et al., 2016; Toljander et al., 2007).

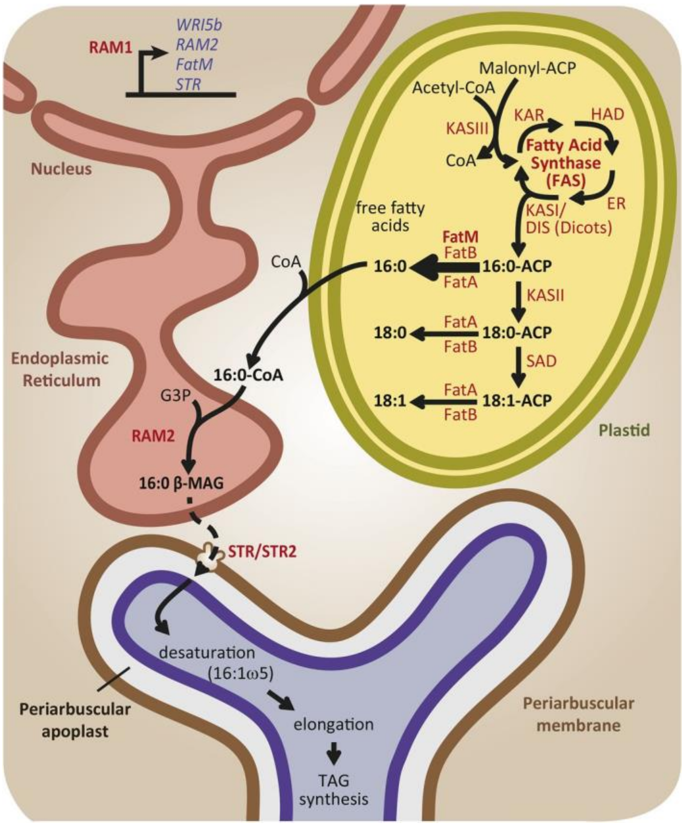


Figure 1.4. Lipid metabolism in the colonized cell and transfer to the arbuscular mycorrhizal fungi (purple colour). Taken from MacLean et al. (2017).

1.3 Motivation and Hypothesis

Thinking of a scenario where there may be a P shortage in the short term, understanding the processes that underlie P mobilisation by AMF will help us reduce the dependency on soluble fertilizers in managed ecosystems, by learning the strategies already employed in natural ecosystems. AMF should not be taken as bio-fertilizers, because they do not add new P to the soil, but they can be used to mine soils for the different soil P fractions (Cardoso and Kuyper, 2006). AMF might contribute to the plant P acquisition from a minor fraction to almost all acquired P (Javot et al., 2007; Lambers et al., 2015), with different abilities to access the various soil P pools. The AMF possess the ability to mobilise OP from the soil solution (Smith et al., 2003), but in in agricultural systems, the excessive application of water-soluble mineral P usually reduces the dependency of plants on P uptake via the mycorrhizal pathway (Jansa et al., 2011). AMF also have the ability to mine P, from recalcitrant sources through solubilizing or hydrolyzing exudates, unavailable for the non-mycorrhizal (Cardoso et al., 2006; Lambers et al., 2008). The AM may increase the availability of organic P sources, mineralizing the organic P through the action of AMF acid phosphatases, which are able to act on phosphate esters (Joner et al., 2000; Koide and Kabir, 2000; Sato et al., 2015; Tarafdar et al., 2002; Tarafdar and Marschner, 1994a, 1994b; Tisserant et al., 2012). In addition, the AMF appear able to dissolve and mobilise the P from the AP primary mineral, through the acidification of the environment (Ness and Vlek, 2000). The inorganic and organic P desorption from the surface of secondary minerals by AMF is not sufficiently understood. The AMF can desorb OP bound to the Fe secondary mineral ferrihydrite (Rakshit and Bhadoria, 2010), but not to GOE, one of the most abundant Fe (oxy)-hydroxide secondary minerals in highly weathered soils (Giles et al. 2012). I am not aware of any study investigating whether AMF, alone or in conjunction with hyphospheric bacteria, has the ability to mobilise OP or PA bound to GOE. The release of P bound to an Fe secondary mineral would require a chelation step by the action of LMWOA (Geelhoed et al., 1999), normally through a process of ligand exchange, whereby the LMWOA (E.g. citrate) directly replaces P on ligand exchange surfaces (Jones, 1998). However, production of LMWOA by AMF is also still poorly documented (Manaut et al., 2014), and thus requires further attention as well.

Raven (2018) suggested that a lower P availability in the soil would require additional support of AMF mycelium biomass to achieve the same P acquisition, resulting in increased construction and maintenance costs for the AM plant. The differing plant P acquisition strategies require C as energy currency (Li et al., 2018), and Turner (2008) hypothesized the different soil P forms can be ranked along a gradient of increasing C investment depending on its accessibility, with PA as the P source most resistant to hydrolysis, and the most metabolically expensive source among the soil P compounds. Wallander and Nylund (1992) and more recently Smits et al. (2012) et al. investigated P nutrition in ectomycorrhizas. Both studies demonstrated in the absence of readily available P and/or under low P conditions, the C allocation into the external mycelium increased proportionally, in order to mobilise the P into the mycorrhizal plant. As Jansa et al (2011) proposed, there may be different strategies for acquiring the different soil P fractions. The P recalcitrant forms, which require the prior action of solubilizing or hydrolyzing exudates, would demand a longer lifespan of the mycelium.

While the mobilisation of easily available P far from the roots, would be carried out in a more efficient way by fungi with a rapid turnover of hyphae, which would expand rapidly in uncolonized soil patches. However, no such studies have yet been carried out for AMF to determine whether inorganic, mineral, organic or P fractions bound to secondary minerals imply a different C cost for the AM plant and this hypothesis remains to be tested experimentally between the different soil P fractions.

After reviewing the most relevant bibliography regarding the functionality of AMF acquiring P for the plant through the symbiotic relationship, some questions remain unanswered regarding the acquisition costs of each of the soil P fractions, as well as the possible mechanisms involved in the mobilisation of the different P forms. In order to deepen our understanding of the C for P trading in AM symbiosis for different forms of P with different accessibility, our work aimed at:

1. Clarifying whether an AM plant can take up P from less accessible sources exclusively through the mycorrhizal pathway,
2. Determining whether a trading mechanism exists between C and P over the AM, when accessing P from sources with differing accessibility,
3. Identifying the mechanisms implemented by the plant and fungus to acquire the different sources of P

The following hypothesis were addressed in the current dissertation:

H1. AM plants accessing the different P sources will mobilise P in different amounts and rates.

H2. The less accessible P sources will require larger photoassimilates investment into the AMF, resulting in differing trading costs per P unit compared to soluble forms.

H3. P mobilisation will enhance the photosynthetic function in case the AM plant access a P source, for maintaining an optimal photosynthetic transfer to the AMF.

H4. The AMF development will correlate positively with P incorporation into the plant tissues for all P sources.

H5. The P mobilisation from the different sources will change the low molecular weight organic acid (LMWOA) profile, with more abundant di/tricarboxylic LMWOA in case of the less accessible P sources.

I performed three separated studies to test the hypothesis. In studies 1 (S1) and 2 (S2), I concentrated on determining whether AMF can mobilise P derived from five sources with potential differing accessibilities. In parallel, I determined the plant investments entailed by the mobilisation of each P source. In the study 3 (S3), I attempted to elucidate the underlying mechanisms behind the mobilisation of the different P forms. A brief description on the material and methods can be found in Figure 1.5:

Study 1

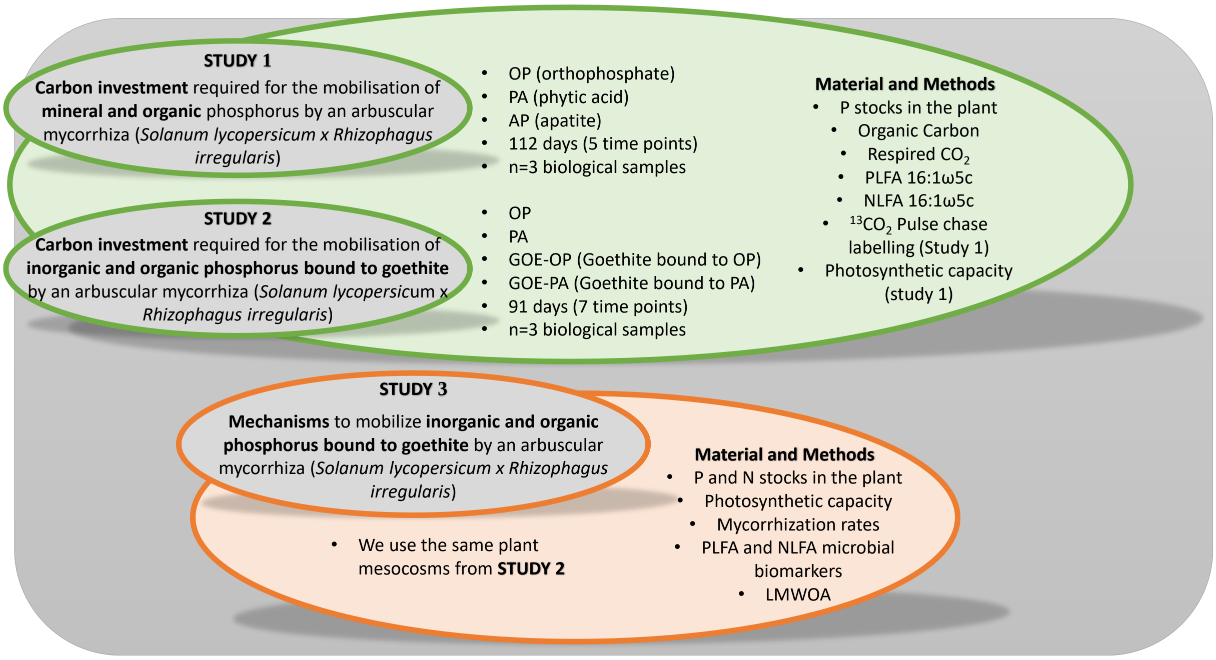
Mesocosms experiments with *Solanum lycopersicum* L. plants mycorrhized with the AMF *Rhizophagus irregularis*, were run in a two-compartment design, with a fungal compartment where P sources were exclusively accessible by the AMF. I offered orthophosphate (OP), apatite (AP) or phytate (PA) as the only P source available to AM symbioses in a mesocosm experiment, where the fungal partner had exclusive access to the respective P source. After exposure, I determined P stocks in the plant tissues, related these to the overall C budget of the system, including a monitoring of the organic C, respired CO_2, the phospholipid fatty acid (PLFA) 16:1ω5c (extraradical mycelium) and the neutral fatty acid (NLFA) 16:1ω5c (energy storage) at the fungal compartment.

Study 2

I used the same mesocosm system as for S1 and compared P uptake rates of AM plants for orthophosphate (OP) and phytic acid (PA), applied to mesocosms in either dissolved form or bound to goethite (α-FeOOH). To estimate the C budget of the symbiosis, I determined total organic carbon (OC), 16:1ω5c phospholipid fatty acid (PLFA; AM fungi extraradical mycelium), 16:1ω5c neutral lipid fatty acid (NLFA; AM fungi energy storage), and CO_2 cumulative respiration in the fungal compartment.

Study 3

I worked with the same mesocosm samples from S2, aiming to elucidate the mechanisms involved in the mobilisation of orthophosphate (OP) or phytic acid (PA) from sorption sites of goethite relative to respective free P sources. In the plant compartment N and P contents, photosynthesis, and mycorrhization rates were determined while at the fungal compartment low-molecular weight organic acids (LMWOA) and fatty acid microbial biomarkers were monitored.



STUDY 1

Carbon investment required for the mobilisation of **mineral and organic** phosphorus by an arbuscular mycorrhiza (*Solanum lycopersicum x Rhizophagus irregularis*)

- OP (orthophosphate)
- PA (phytic acid)
- AP (apatite)
- 112 days (5 time points)
- n=3 biological samples

STUDY 2

Carbon investment required for the mobilisation of **inorganic and organic phosphorus bound to goethite** by an arbuscular mycorrhiza (*Solanum lycopersicum x Rhizophagus irregularis*)

- OP
- PA
- GOE-OP (Goethite bound to OP)
- GOE-PA (Goethite bound to PA)
- 91 days (7 time points)
- n=3 biological samples

Material and Methods

- P stocks in the plant
- Organic Carbon
- Respired CO_2
- PLFA 16:1ω5c
- NLFA 16:1ω5c
- $^{13}CO_2$ Pulse chase labelling (Study 1)
- Photosynthetic capacity (study 1)

STUDY 3

Mechanisms to mobilize **inorganic and organic phosphorus bound to goethite** by an arbuscular mycorrhiza (*Solanum lycopersicum x Rhizophagus irregularis*)

- We use the same plant mesocosms from **STUDY 2**

Material and Methods

- P and N stocks in the plant
- Photosynthetic capacity
- Mycorrhization rates
- PLFA and NLFA microbial biomarkers
- LMWOA

Figure 1.5. Overview of the material and methods used in each study.

1.4 References

Agnolucci, M., Battini, F., Cristani, C., and Giovannetti, M. (2015). Diverse bacterial communities are recruited on spores of different arbuscular mycorrhizal fungal isolates. *Biol. Fertil. Soils* 51, 379–389. doi:10.1007/s00374-014-0989-5.

Arif, M. S., Shahzad, S. M., Yasmeen, T., Riaz, M., Ashraf, M., Ashraf, M. A., et al. (2017). “Improving Plant Phosphorus (P) Acquisition by Phosphate-Solubilizing Bacteria,” in *Essential Plant Nutrients* (Cham: Springer International Publishing), 513–556. doi:10.1007/978-3-319-58841-4.

Bago, B., Zipfel, W., Williams, R. M., Jun, J., Arreola, R., Lammers, P. J., et al. (2002). Translocation and utilization of fungal storage lipid in the arbuscular mycorrhizal symbiosis. *Plant Physiol.* 128, 108–124. doi:10.1104/pp.010466.

Becquer, A., Trap, J., Irshad, U., Ali, M. A., and Claude, P. (2014). From soil to plant, the journey of P through trophic relationships and ectomycorrhizal association. *Front. Plant Sci.* 5, 548. doi:10.3389/fpls.2014.00548.

Boldt, K., Pörs, Y., Haupt, B., Bitterlich, M., Kühn, C., Grimm, B., et al. (2011). Photochemical processes, carbon assimilation and RNA accumulation of sucrose transporter genes in tomato arbuscular mycorrhiza. *J. Plant Physiol.* 168, 1256–1263. doi:10.1016/j.jplph.2011.01.026.

Bravo, A., Brands, M., Wewer, V., Dörmann, P., and Harrison, M. J. (2017). Arbuscular mycorrhiza-specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote development of arbuscular mycorrhiza. *New Phytol.* 214, 1631–1645. doi:10.1111/nph.14533.

Brundrett, M., Bougher, N., Dell, B., Grove, T., and Malajczuk, N. (1996). *Working with mycorrhizas in forestry and agriculture*. Canberra: Australian Centre for International Agricultural Research.

Bünemann, E. K., Prusisz, B., and Ehlers, K. (2011). “Characterization of Phosphorus Forms in Soil Microorganisms,” in, 37–57. doi:10.1007/978-3-642-15271-9_2.

Cardoso, I. M., Boddington, C. L., Janssen, B. H., Oenema, O., and Kuyper, T. W. (2006). Differential Access to Phosphorus Pools of an Oxisol by Mycorrhizal and Nonmycorrhizal Maize. *Commun. Soil Sci. Plant Anal.* 37, 1537–1551. doi:10.1080/00103620600710074.

Cardoso, I. M., and Kuyper, T. W. (2006). Mycorrhizas and tropical soil fertility. 116, 72–84. doi:10.1016/j.agee.2006.03.011.

Cheng, L., Booker, F., Tu, C., and Burkey, K. (2012). Arbuscular mycorrhizal fungi increase organic carbon decomposition under elevated CO2. *Science (80-.).* Available at: http://www.sciencemag.org/content/337/6098/1084.short [Accessed October 9, 2015].

Choudhary, D. K., Varma, A., and Tuteja, N. (2017). "Mycorrhizal Helper Bacteria: Sustainable Approach," in *Mycorrhiza - Function, Diversity, State of the Art* (Cham: Springer International Publishing), 61–74. doi:10.1007/978-3-319-53064-2_5.

Cross, A. F., and Schlesinger, W. H. (1995). A literature review and evaluation of the. Hedley fractionation: Applications to the biogeochemical cycle of soil phosphorus in natural ecosystems. *Geoderma* 64, 197–214. doi:10.1016/0016-7061(94)00023-4.

Dalpé, Y., Trépanier, M., Sahraoui, A. L.-H., Fontaine, J., and Sancholle, M. (2012). Lipids of Mycorrhizas. *Fungal Assoc.* 9, 137–169. doi:10.1007/978-3-642-30826-0_8.

Devau, N., Cadre, E. Le, Hinsinger, P., Jaillard, B., and Gérard, F. (2009). Soil pH controls the environmental availability of phosphorus: Experimental and mechanistic modelling approaches. *Appl. Geochemistry* 24, 2163–2174. doi:10.1016/J.APGEOCHEM.2009.09.020.

Elser, J., and Bennett, E. (2011). A broken biogeochemical cycle. *Nature* 478, 29–31. doi:10.1038/478029a.

Geelhoed, J. S., Van Riemsdijk, W. H., and Findenegg, G. R. (1999). Simulation of the effect of citrate exudation from roots on the plant availability of phosphate adsorbed on goethite. *Eur. J. Soil Sci.* 50, 379–390. doi:10.1046/j.1365-2389.1999.00251.x.

George, T. S., Fransson, A.-M., Hammond, J. P., and White, P. J. (2011). "Phosphorus Nutrition: Rhizosphere Processes, Plant Response and Adaptations," in (Springer, Berlin, Heidelberg), 245–271. doi:10.1007/978-3-642-15271-9_10.

Gianinazzi, S., Gollotte, A., Binet, M. N., van Tuinen, D., Redecker, D., and Wipf, D. (2010). Agroecology: The key role of arbuscular mycorrhizas in ecosystem services. *Mycorrhiza* 20, 519–530. doi:10.1007/s00572-010-0333-3.

Gilbert, N. (2009). Environment: The disappearing nutrient. *Nature* 461, 716–8. doi:10.1038/461716a.

Giles, C. D., Richardson, A. E., Druschel, G. K., and Hill, J. E. (2012). Organic anion-driven solubilization of precipitated and sorbed phytate improves hydrolysis by phytases and bioavailability to Nicotiana tabacum. *Soil Sci.* 177, 591–598. doi:10.1097/SS.0b013e318272f83f.

Graham, J. H., Hodge, N. C., Morton, J. B. B., Graham, J. H., and Hodge, N. C. (1995). Fatty Acid methyl ester profiles for characterization of glomalean fungi and their These include : Fatty Acid Methyl Ester Profiles for Characterization of Glomalean Fungi and Their

Endomycorrhizae †. *Appl. Environ. Microbiol.* 61, 58–64.

Gui, H., Purahong, W., Hyde, K. D., Xu, J., and Mortimer, P. E. (2017). The arbuscular mycorrhizal fungus Funneliformis mosseae alters bacterial communities in subtropical forest soils during litter decomposition. *Front. Microbiol.* 8, 1120. doi:10.3389/fmicb.2017.01120.

Herder, G. Den, Van Isterdael, G., Beeckman, T., and De Smet, I. (2010). The roots of a new green revolution. *Trends Plant Sci.* 15, 600–607. doi:10.1016/j.tplants.2010.08.009.

Hodge, A. (2016). "Accessibility of Inorganic and Organic Nutrients for Mycorrhizas," in *Mycorrhizal Mediation of Soil: Fertility, Structure, and Carbon Storage* (Elsevier), 129–148. doi:10.1016/B978-0-12-804312-7.00008-5.

Jansa, J., Finlay, R. D., Wallander, H., Smith, F. A., and Smith, S. E. (2011). "Role of Mycorrhizal Symbioses in Phosphorus Cycling," in *Phosphorus in Action. Biological Processes in Soil Phosphorus Cycling*, 137–168. doi:10.1007/978-3-642-15271-9.

Javaid, A. (2009). Arbuscular Mycorrhizal Mediated Nutrition in Plants. *J. Plant Nutr.* 32, 1595–1618. doi:10.1080/01904160903150875.

Javot, H., Pumplin, N., and Harrison, M. J. (2007). Phosphate in the arbuscular mycorrhizal symbiosis: transport properties and regulatory roles. *Plant. Cell Environ.* 30, 310–22. doi:10.1111/j.1365-3040.2006.01617.x.

Jiang, Y., Wang, W., Xie, Q., Liu, N., Liu, L., Wang, D., et al. (2017). Plants transfer lipids to sustain colonization by mutualistic mycorrhizal and parasitic fungi. *Science (80-.).* 356, 1172–1173. doi:10.1126/science.aam9970.

Johnson, N. C. (2010). Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New Phytol.* 185, 631–647. doi:10.1111/j.1469-8137.2009.03110.x.

Joner, E. J., Van Aarle, I. M., and Vosatka, M. (2000). Phosphatase activity of extra-radical arbuscular mycorrhizal hyphae: A review. in *Plant and Soil*, 199–210. doi:10.1023/A:1026582207192.

Jones, D. L. (1998). Organic acids in the rhizosphere - A critical review. *Plant Soil* 205, 25–44. doi:10.1023/A:1004356007312.

Kaiser, C., Kilburn, M. R., Clode, P. L., Fuchslueger, L., Koranda, M., Cliff, J. B., et al. (2015). Exploring the transfer of recent plant photosynthates to soil microbes: Mycorrhizal pathway vs direct root exudation. *New Phytol.* 205, 1537–1551. doi:10.1111/nph.13138.

Keymer, A., and Gutjahr, C. (2018). Cross-kingdom lipid transfer in arbuscular mycorrhiza symbiosis and beyond. *Curr. Opin. Plant Biol.* 44, 137–144.

doi:10.1016/j.pbi.2018.04.005.

Keymer, A., Pimprikar, P., Wewer, V., Huber, C., Brands, M., Bucerius, S. L., et al. (2017). Lipid transfer from plants to arbuscular mycorrhiza fungi. *Elife* 6. doi:10.7554/eLife.29107.

Koide, R. T. (1991). Nutrient supply, nutrient demand and plant response to mycorrhizal infection. *New Phytol.* 117, 365–386. doi:10.1111/j.1469-8137.1991.tb00001.x.

Koide, R. T., and Kabir, Z. (2000). Extraradical hyphae of the mycorrhizal fungus Glomus intraradices can hydrolyse inorganic phosphate. *New Phytol.* 148, 511–517. doi:10.1046/j.1469-8137.2000.00776.x.

Krishnamoorthy, R., Kim, K., Subramanian, P., Senthilkumar, M., Anandham, R., and Sa, T. (2016). Arbuscular mycorrhizal fungi and associated bacteria isolated from salt-affected soil enhances the tolerance of maize to salinity in coastal reclamation soil. *Agric. Ecosyst. Environ.* 231, 233–239. doi:10.1016/j.agee.2016.05.037.

Lambers, H., Martinoia, E., and Renton, M. (2015). Plant adaptations to severely phosphorus-impoverished soils. *Curr. Opin. Plant Biol.* 25, 23–31. doi:10.1016/j.pbi.2015.04.002.

Lambers, H., Raven, J. A., Shaver, G. R., and Smith, S. E. (2008). Plant nutrient-acquisition strategies change with soil age. *Trends Ecol. Evol.* 23, 95–103. doi:10.1016/j.tree.2007.10.008.

Leake, J. R., and Read, D. J. (2016). “Mycorrhizal Symbioses and Pedogenesis Throughout Earth’s History,” in *Mycorrhizal Mediation of Soil: Fertility, Structure, and Carbon Storage* (Elsevier), 9–33. doi:10.1016/B978-0-12-804312-7.00002-4.

Li, C., Kuyper, T. W., van der Werf, W., Zhang, J., Li, H., Zhang, F., et al. (2018). Testing for complementarity in phosphorus resource use by mixtures of crop species. *Plant Soil*, 1–15. doi:10.1007/s11104-018-3732-4.

Luginbuehl, L. H., Menard, G. N., Kurup, S., Van Erp, H., Radhakrishnan, G. V, Breakspear, A., et al. (2017). Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant. *Science* 356, 1175–1178. doi:10.1126/science.aan0081.

MacLean, A. M., Bravo, A., and Harrison, M. J. (2017). Plant signaling and metabolic pathways enabling arbuscular mycorrhizal symbiosis. *Plant Cell* 29, tpc.00555.2017. doi:10.1105/tpc.17.00555.

Manaut, N., Sanguin, H., Ouahmane, L., Bressan, M., Thioulouse, J., Baudoin, E., et al. (2014). Potentialities of ecological engineering strategy based on native arbuscular mycorrhizal community for improving afforestation programs with carob trees in degraded environments. *Ecol. Eng.* 79, 113–119. doi:10.1016/j.ecoleng.2015.03.007.

Mishra, P., Joshi, P., and Suyal, P. (2014). “Potential of phosphate solubilising microorganisms in crop production,” in *Bioresources for Sustainable Plant Nutrient Management* (Satish Serial Publishing House), 201–222.

Ness, R. L. L. L., and Vlek, P. L. G. G. (2000). Mechanism of calcium and phosphate release from hydroxy-apatite by mycorrhizal hyphae. *Soil Sci. Soc. Am. J.* 64, 949–955. doi:10.2136/sssaj2000.643949x.

Nottingham, A. T., Turner, B. L., Winter, K., van der Heijden, M. G. A., and Tanner, E. V. J. (2010). Arbuscular mycorrhizal mycelial respiration in a moist tropical forest. *New Phytol.* 186, 957–967. doi:10.1111/j.1469-8137.2010.03226.x.

Nuccio, E. E., Hodge, A., Pett-Ridge, J., Herman, D. J., Weber, P. K., and Firestone, M. K. (2013). An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. *Environ. Microbiol.* 15, 1870–1881. doi:10.1111/1462-2920.12081.

Olsson, P. A., and Johansen, A. (2000). Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. *Mycol. Res.* 104, 429–434. doi:10.1017/S0953756299001410.

Olsson, P. A., and Johnson, N. C. (2005). Tracking carbon from the atmosphere to the rhizosphere. *Ecol. Lett.* 8, 1264–1270. doi:10.1111/j.1461-0248.2005.00831.x.

Osorio, N. W., and Habte, M. (2001). Synergistic Influence of an Arbuscular Mycorrhizal Fungus and a P Solubilizing Fungus on Growth and P Uptake of Leucaena leucocephala in an Oxisol. *Arid L. Res. Manag.* 15, 263–274. doi:10.1080/15324980152119810.

Parniske, M. (2008). Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat. Rev. Microbiol.* 6, 763–75. doi:10.1038/nrmicro1987.

Paterson, E., Sim, A., Davidson, J., and Daniell, T. J. (2016). Arbuscular mycorrhizal hyphae promote priming of native soil organic matter mineralisation. *Plant Soil* 408, 243–254. doi:10.1007/s11104-016-2928-8.

Pel, R., Dupin, S., Schat, H., Ellers, J., Kiers, E. T., and van Straalen, N. M. (2018). Growth benefits provided by different arbuscular mycorrhizal fungi to Plantago lanceolata depend on the form of available phosphorus. *Eur. J. Soil Biol.* 88, 89–96. doi:10.1016/j.ejsobi.2018.07.004.

Pellegrino, E., and Bedini, S. (2014). Enhancing ecosystem services in sustainable agriculture: Biofertilization and biofortification of chickpea (Cicer arietinum L.) by arbuscular mycorrhizal fungi. *Soil Biol. Biochem.* 68, 429–439. doi:10.1016/j.soilbio.2013.09.030.

Rakshit, A., and Bhadoria, pratapbhanu (2010). Role of VAM on growth and phosphorus nutrition of maize with low soluble phosphate fertilization. *Acta Agronómica* 59, 119–123. Available at: http://www.revistas.unal.edu.co/index.php/acta_agronomica/article/view/14101/14960.

Raven, J. A., Lambers, H., Smith, S. E., and Westoby, M. (2018). Costs of acquiring phosphorus by vascular land plants: patterns and implications for plant coexistence. *New Phytol.* 217, 1420–1427. doi:10.1111/nph.14967.

Richardson, A. E., Hocking, P. J., Simpson, R. J., and George, T. S. (2009). Plant mechanisms to optimise access to soil phosphorus. *Crop Pasture Sci.* 60, 124–143. doi:10.1071/CP07125.

Rowe, H., Withers, P. J. A., Baas, P., Chan, N. I., Doody, D., Holiman, J., et al. (2016). Integrating legacy soil phosphorus into sustainable nutrient management strategies for future food, bioenergy and water security. *Nutr. Cycl. Agroecosystems* 104, 393–412. doi:10.1007/s10705-015-9726-1.

Ryan, M. H., Tibbett, M., Edmonds-Tibbett, T., Suriyagoda, L. D. B., Lambers, H., Cawthray, G. R., et al. (2012). Carbon trading for phosphorus gain: The balance between rhizosphere carboxylates and arbuscular mycorrhizal symbiosis in plant phosphorus acquisition. *Plant, Cell Environ.* 35, 2170–2180. doi:10.1111/j.1365-3040.2012.02547.x.

Sato, T., Ezawa, T., Cheng, W., and Tawaraya, K. (2015). Release of acid phosphatase from extraradical hyphae of arbuscular mycorrhizal fungus Rhizophagus clarus. *Soil Sci. Plant Nutr.* 61, 269–274. doi:10.1080/00380768.2014.993298.

Schachtman, D. P., Reid, R. J., and Ayling, S. M. (1998). Update on Phosphorus Uptake Phosphorus Uptake by Plants: From Soil to Cell. 447–453. doi:10.1104/pp.116.2.447.

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., et al. (2011). Phosphorus Dynamics: From Soil to Plant. *Plant Physiol.* 156, 997–1005. doi:10.1104/pp.111.175232.

Slavikova, R., Püschel, D., Janouskova, M., Hujslova, M., Konvalinkova, T., Gryndlerova, H., et al. (2016). Monitoring CO2 emissions to gain a dynamic view of carbon allocation to arbuscular mycorrhizal fungi. *Mycorrhiza* 27, 1–17. doi:10.1007/s00572-016-0731-2.

Smith, F. A., and Smith, S. E. (2011). What is the significance of the arbuscular mycorrhizal colonisation of many economically important crop plants? *Plant Soil* 348, 63–79. doi:10.1007/s11104-011-0865-0.

Smith, S. E., and Read, D. J. (2008). *Mycorrhizal symbiosis*. Academic Press doi:10.1016/B978-0-12-370526-6.X5001-6.

Smith, S. E., and Smith, F. A. (2012). Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia* 104, 1–13. doi:10.3852/11-229.

Smith, S. E., Smith, F. A., and Jakobsen, I. (2003). Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiol.* 133, 16–20. doi:10.1104/pp.103.024380.

Smits, M. M., Bonneville, S., Benning, L. G., Banwart, S. A., and Leake, J. R. (2012). Plant-driven weathering of apatite - the role of an ectomycorrhizal fungus. *Geobiology* 10, 445–456. doi:10.1111/j.1472-4669.2012.00331.x.

Solaiman, M. Z., Ezawa, T., Kojima, T., and Saito, M. (1999). Polyphosphates in intraradical and extraradical hyphae of an arbuscular mycorrhizal fungus, Gigaspora margarita. *Appl. Environ. Microbiol.* 65, 5604–5606.

Staddon, P. L., Ramsey, C. B., Ostle, N., Ineson, P., and Fitter, A. H. (2003). Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of 14C. *Science* 300, 1138–40. doi:10.1126/science.1084269.

Stürmer, S. L., Bever, J. D., and Morton, J. B. (2018). Biogeography of arbuscular mycorrhizal fungi (Glomeromycota): a phylogenetic perspective on species distribution patterns. *Mycorrhiza* 28, 587–603. doi:10.1007/s00572-018-0864-6.

Tarafdar, J. C., and Marschner, H. (1994a). Efficiency of VAM hyphae in utilisation of organic phosphorus by wheat plants. *Soil Sci. Plant Nutr.* 40, 593–600. doi:10.1080/00380768.1994.10414298.

Tarafdar, J. C., and Marschner, H. (1994b). Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. *Soil Biol. Biochem.* 26, 387–395. doi:10.1016/0038-0717(94)90288-7.

Tarafdar, J. C., Yadav, R. S., and Niwas, R. (2002). Relative efficiency of fungal intra- and extracellular phosphatases and phytase. *J. Plant Nutr. Soil Sci.* 165, 17–19. doi:10.1002/1522-2624(200202)165:1<17::AID-JPLN17>3.0.CO;2-C.

Tiessen, H., Ballester, M. V., and Salcedo, I. (2011). “Phosphorus and Global Change,” in, 459–471. doi:10.1007/978-3-642-15271-9_18.

Tisserant, E., Kohler, A., Dozolme-Seddas, P., Balestrini, R., Benabdellah, K., Colard, A., et al. (2012). The transcriptome of the arbuscular mycorrhizal fungus Glomus intraradices (DAOM 197198) reveals functional tradeoffs in an obligate symbiont. *New Phytol.* 193, 755–769. doi:10.1111/j.1469-8137.2011.03948.x.

Toljander, J. F., Lindahl, B. D. B. D., Paul, L. R., Elfstrand, M., and Finlay, R. D. (2007). Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. *FEMS Microbiol. Ecol.* 61, 295–304. doi:10.1111/j.1574-6941.2007.00337.x.

Tomè, E., Ventura, M., Folegot, S., Zanotelli, D., Montagnani, L., Mimmo, T., et al. (2016). Mycorrhizal contribution to soil respiration in an apple orchard. *Appl. Soil Ecol.* 101, 165–173. doi:10.1016/j.apsoil.2016.01.016.

Trépanier, M., Bécard, G., Moutoglis, P., Willemot, C., Gagné, S., Avis, T. J., et al. (2005). Dependence of Arbuscular-Mycorrhizal Fungi on Their Plant Host for Palmitic Acid Synthesis Dependence of Arbuscular-Mycorrhizal Fungi on Their Plant Host for Palmitic Acid Synthesis. *Society* 71, 5341–5347. doi:10.1128/AEM.71.9.5341.

Turner, B. L. (2008). Resource partitioning for soil phosphorus: A hypothesis. *J. Ecol.* 96, 698–702. doi:10.1111/j.1365-2745.2008.01384.x.

Walker, T. W., and Syers, J. K. (1976). The fate of phosphorus during pedogenesis. *Geoderma* 15, 1–19. doi:10.1016/0016-7061(76)90066-5.

Wallander, H., and Nylund, J. (1992). Effects of excess nitrogen and phosphorus starvation on the extramatrical mycelium of ectomycorrhizas of Pinus sylvestris L. *New Phytol.* 120, 495–503. doi:10.1111/j.1469-8137.1992.tb01798.x.

Willis, A., Rodrigues, B. F., and Harris, P. J. C. (2013). The Ecology of Arbuscular Mycorrhizal Fungi. *CRC. Crit. Rev. Plant Sci.* 32, 1–20. doi:10.1080/07352689.2012.683375.

Yang, X., Post, W. M., Thornton, P. E., and Jain, A. (2013). The distribution of soil phosphorus for global biogeochemical modeling. *Biogeosciences* 10, 2525–2537. doi:10.5194/bg-10-2525-2013.

Zhu, Y. G., and Miller, R. M. (2003). Carbon cycling by arbuscular mycorrhizal fungi in soil-plant systems. *Trends Plant Sci.* 8, 407–409. doi:10.1016/S1360-1385(03)00184-5.

2. Study I

Carbon investment required for the mobilisation of mineral and organic phosphorus by an arbuscular mycorrhiza (*Solanum lycopersicum x Rhizophagus irregularis*)

Alberto Andrino[1], Jens Boy[1], Leopold Sauheitl[1], Stefan Burkart[2], Reinhard Well[2] Cornelia Schneider[1] and Georg Guggenberger[1]

[1]Institute of Soil Science, Leibniz Universität Hannover, Herrenhäuser Straße 2, 30419 Hannover, Germany.
[2]Institute of Climate Smart Agriculture, Thünen-Institut, Bundesallee 65, 38116 Braunschweig, Germany.

Author contribution

JB, GG, AA and CS designed the experiment. AA and CS prepared the plant and fungal material. SB, RW and AA carried out the Picarro measurements at the Institute of Climate Smart Agriculture facilities. AA conducted the experiment and analysed the data. JB, LS and GG supervised the research. AA wrote the paper with contributions from JB, GG, LS, SB and RW.

Prepared for submission

Abstract

Phosphorus (P) is a limiting element in most ecosystems and around 80% of plant species establish symbiosis with arbuscular mycorrhizal fungi (AMF) to overcome the P deficiency, which in return constitute a major sink of photosynthates. Soil P forms in soil differ in their acquisition costs for the symbiosis, with insoluble inorganic and organic P-forms being the most metabolically expensive, thus requiring the highest investments of photoassimilates by the plant. So far, it stayed unclear whether C allocation towards AMF increases with declining availability of P-sources, the hypothesis we aim to test by this study. We offered each of orthophosphate (OP), apatite (AP) or phytate (PA) as the only P source available to AM symbioses in a mesocosm experiment, where the fungal partner had exclusive access to the respective P source. After exposure, we determined P stocks in the plant tissues, related these to the overall C budget of the system, including the organic carbon (C), respired CO_2, the phospholipid fatty acid (PLFA) 16:1ω5c (extraradical mycelium) and the neutral fatty acid (NLFA) 16:1ω5c (energy storage) at the fungal compartment. Arbuscular mycorrhizal plants incorporated P derived from the three P sources through the mycorrhizal pathway, but did this with differing C-P trading costs. The mobilisation of PA and AP by the AM plant entailed larger mycelium infrastructure and significantly higher respiratory losses of CO_2, in comparison to the utilization the OP. Our study suggest, AM plants investing in a tight coupling larger amounts of C into their fungal partners at lower P availability. This larger C flow to the AM fungi might also lead to larger soil organic C contents.

Keywords

Apatite, organic phosphorus, phytate, inorganic phosphorus, arbuscular mycorrhiza, PLFA 16:1ω5c, NLFA 16:1ω5c, photosynthesis, Picarro.

Introduction

Phosphorus (P) plays an important role in plant growth and development. It is the most limiting nutrient for plant growth after nitrogen (N) (Khosravi et al. 2017). In natural ecosystems, the primary source of P is provided by mineral weathering (Walker and Syers 1976). The most important primary mineral in this process is apatite which accounts for over 95% of all P in the Earth's crust, thus playing a vital role in providing P to all ecosystems (Smits et al. 2012). Over time, the phosphate ions released by weathering have been partially incorporated into soil, plants and microbes, becoming part of the organic P pool (Arcand and Schneider 2006). Also, phosphate released by weathering or from enzymatic decomposition of organic P species can be adsorbed to Fe- and Al- (hydr)oxides in acidic soils, while in calcareous soils it is found in form of sparingly soluble Ca phosphates (Li et al. 2018).

For the abovementioned reasons, P is often a limiting resource in natural and managed ecosystems and, as a consequence, almost 80% of plant species establish symbiotic associations with arbuscular mycorrhizal fungi (AMF) (Smith and Read 2008; Ryan et al. 2012). This symbiotic relationship, being several hundreds of millions years old (Lambers et al. 2009), explores a large volume of soil and does this at a lower cost as compared to roots. Such low-cost modes of P acquisition are expected to be favoured by natural selection and during the assembly of plant communities by competition (Raven et al. 2018) and AMF's hyphae represents the lower construction and maintenance costs infrastructure (2-20 μm diameter), compared to root hair development (100-500 μm diameter) (Hodge 2016; Leake and Read 2016). AMF are able to mobilise the otherwise poorly accessible phosphate ions beyond the P depletion zone (Zhang et al. 2016; Osorio et al. 2017), and are responsible for nearly 80% of the plant P uptake (Kahiluoto and Vestberg 1998; Douds and Johnson 2007; Řezáčová et al. 2017). Mycorrhizal benefits are greater under P-limiting soil conditions than in P-rich ones. In exchange for the P acquisition, AMF represents a major sink for recently fixed plant photosynthates (Nottingham et al. 2010), deriving the host plant C into their growth, respiration, and biological maintenance (Jansa et al. 2011). Arbuscular mycorrhizal plants allocate 4-20% of the total C fixed into the AMF structures (Parniske 2008), and between 1 and 5% of net photosynthesis is lost by hyphal respiration (Tomè et al. 2016). AMF may also act as a gateway for direct delivery of recent plant photosynthates to the hyphospheric microbes within hours (Kaiser et al. 2015), exhibiting an intimate relationship and suggesting a synergistic cooperation (Jansa et al. 2011; Zhang et al. 2016).

All plant acquisition strategies of P require photoassimilates as energy currency (Li et al. 2018). In this respect, Turner (2008) hypothesized the different soil P forms can be placed along a gradient of increasing energy investment required for their acquisition, since insoluble organic and inorganic P forms have comparatively high pre-processing costs, even at high concentrations, and these costs also rise steeply as concentration declines (Raven et al. 2018). Organic P constitutes 20-80 % of the total soil, with phytic acid (PA) representing >50% of the organic P (Shen et al. 2011). Turner (2008) identified PA as the P source most resistant to hydrolysis, and the most metabolically expensive source among the soil P compounds. For ectomycorrhizas, it has been already demonstrated that the C allocation into the external mycelium increases proportionally at absence of readily available P and/or under low P conditions, (Smits et al. 2012). To our knowledge, such studies are absent for AMF. Raven

(2018) hypothesized that a greater length of AMF mycelium needs to be supported at low P availability in order to compensate, resulting in increased construction and maintenance costs of the mycelium (Raven et al. 2018)**.**

Our work aimed at determining whether AMF can mobilise P from mineral and organic sources while determining the acquisition costs imposed by the mobilisation of each of them in terms of photoassimilate investments by the plant in parallel. For this purpose, we chose AP and PA for a comparison. AMF appear to be able to enter AP primary mineral structures through the exudation of acids (Ness and Vlek 2000). Comparable processes were observed in numerous studies with ectomycorrhizal fungi that could dissolve minerals through organic acid excretion (Rosling et al. 2004; Smits et al. 2008, 2012). AMF may be able to mineralize organic P forms through the action of their own acid phosphatases and access P sources that non-mycorrhizal plants are unable to mobilise (Tarafdar and Marschner 1994a, b; Joner et al. 2000; Koide and Kabir 2000; Tarafdar et al. 2002; Tisserant et al. 2012; Steidinger et al. 2015; Sato et al. 2015). In order to compare the acquisition costs of incorporating AP and the PA through the mycorrhizal pathway, we have to quantify the plant C investments into the AMF. To that end, we used a mesocosms, where exclusively the AMF hyphae had access to the different P sources in a fungal compartment. We estimated the full C budget of the mycorrhizal symbiosis by measuring the respired CO_2, plus the total organic C accumulated at the fungal compartment. We estimated the AMF extraradical mycelium using the phospholipid fatty acid (PLFA) 16:1ω5c (Olsson and Johansen 2000; Aarle and Olsson 2003; Marschner 2007) and the AMF energy storage by means of the neutral fatty acid (NLFA) 16:1ω5c (Olsson and Wilhelmsson 2000; Aarle and Olsson 2003; Olsson and Johnson 2005), both measured within the fungal compartment. We hypothesized that less accessible P sources, PA and AP, will cause larger photoassimilate investments by the host plant and will result in differing trading costs per P unit in comparison to easily accessible P sources such orthophosphate (OP).

Material and Methods

To compare the acquisition costs of incorporating P from differing accessibility sources through the mycorrhizal pathway, we conducted two experiments. Experiment 1 allowed us to test the ability of the AMF mobilising P from the different sources offered at the fungal compartment during a time course experiment over xyz days, but more importantly, to estimate whether there is a differentiated plant C investment into the AMF. Experiment 2 was carried out to compare the current acquisition costs in form of recent photoassimilates using stable isotope ^{13}C at the specific time point elucidated in the Experiment 1. This was done only at treatments, where it was possible to quantify the incorporation of the different P sources.

Experiment 1

Solanum lycopersicum L. Inoculation with *Rhizophagus irregularis DAOM 197198*

Tomato (*Solanum lycopersicum* L.) seeds var. Moneymaker (Volmary GmbH) were surface sterilized with H_2O_2 (5%, 10 min), rinsed with sterile distilled water (3x) and germinated on the surface of a moistened filter paper inside a petri dish (3 days, 27 ºC). The *R. irregularis* DAOM 197198 model organism (Daubois et al. 2016), was selected as mycorrhizal inoculum due to its ubiquitous occurrence and adaptation to agricultural practices (Köhl et al. 2016). The strain DAOM 197198 consisting of spores and root fragments (Symplanta GmbH & Co. KG. Darmstadt, Germany) was reproduced in a *Sorghum bicolor* trap plant culture (Brundrett et al. 1996). Tomato germinated seeds were sown on 75 ml pots QP96 (HerkuPlast Kubern GmbH) containing 0.4 g AMF and 70 ml autoclaved acid washed quartz sand. The quartz sand was used as plant and fungal growth substrate due to its suitability for the AM mycelium development (Johansen et al. 1996; Olsson and Johansen 2000). Mycorrhizal and control plants were grown at a greenhouse for four weeks (16/8 light/dark, 24/20°C light/dark, 50–60% relative humidity, photon flux density of 175–230 µmol/m^2/s). Plants were watered every day with 10 ml deionized water and on alternate days the pots were fertilized with 5 ml low P (0.32 mM) modified Long Ashton nutrient solution pH 6.5 (Hewitt 1966). A quality control was carried out before the transplantation into the mesocosm, to monitor whether the AM plants were mycorrhized. Tomato roots from AM plants and controls were stained using the ink and vinegar staining technique for visualizing the AMF colonization (Vierheilig et al. 1998b).

Time Course Experiment

The time course experiment consisted of 5 harvesting points and lasted for 112 days. The first harvest point was at the day of transplanting (day 0) to determine initial plant P content (n=5), followed by four sampling points at days 35, 56, 86 and 112. In each harvest point, three biological replicates of each treatment were harvested. Four weeks old mycorrhized and not mycorrhized tomato seedlings were planted into each mesocosm and placed at a greenhouse (24/20°C light/dark; photoperiod 16/8 h light/dark; 50-60% relative humidity; photon flux density of 175–230 µmol/m^2/s). Once a week, mesocosms were rotated within the greenhouse to achieve homogeneous growth conditions.

The mesocosms consisted of a plant compartment and a fungal compartment, into which only the AMF was able to grow and mobilise the P sources (Figure 1). Between both compartments, two barriers separated mycorrhizal roots and mycelium, a 20 µm pore size polyamide mesh (Franz Eckert GmbH) (Watkins et al. 1996; Fitter et al. 1998) and a polytetrafluoroethylene (PTFE) membrane with a pore size of 5-10 µm (Pieper Filter GmbH). The PTFE membrane allowed the AM hyphae to cross but did not allow mass flow and diffusion of ions to the PC, thus sources were only accessible to the AM plant by the hyphae (Mäder et al. 1993, 2000; Vierheilig et al. 1998a).

Phosphorus Sources Offered at the Fungal Compartment

Three P sources were offered at the fungal compartment. Orthophosphate (OP) was used as inorganic and readily available source in form of KH_2PO_4. Phytic acid (PA) (Sigma Aldrich, Taufkirchen, Germany) was chosen as the organic source. A total content of 10 mg P were applied in the form of OP or PA and dissolved in 16 ml autoclaved MilliQ water and mixed with 60 g autoclaved acid washed quartz sand placed inside each fungal compartment. The 10 mg P content was decided on the basis to sustain the plant P necessities during the next 3 months after planting the seedlings into the mesocosms. As third P source, we chose apatite (AP) as the primary mineral source (2 mm diameter) (Krantz Company, Bonn, Germany). An average P content 18.72% within the AP was characterized using a field emission ESEM (Phillips/FEI XL-30, Hillsboro, Oregon, USA) equipped with an energy-dispersive spectrometer for micro-chemical analysis. Easily available P in water was determined for the AP, shaking 1 g of the mineral with 30 ml of autoclaved MilliQ water pH 7 (n=3) into 50 ml falcon test tube. Tubes were placed on an orbital shaker at 250 rpm for 7 days to determine maximum amount of P released from the AP in MilliQ water. The centrifuged supernatant (3000 × *g*, 15 min) was filtered through a 0.45 µm syringe filter (PVDF) and 1 ml was mixed with 1 ml 30% HNO_3 and the mixture was filled up to 10 ml with MilliQ water. Total P concentration was measured by ICP-MS Agilent 7500C (Agilent Technologies Ireland Ltd., Cork, Ireland) and resulted in 0.03 mg +/- 0.004 mg P g^{-1}AP in the MilliQ water. An amount of 3 g containing 562 mg P (0.09 mg easily available P) was added to 60 g quartz sand and 16 ml autoclaved MilliQ water. The purpose of including AP in a greater content compared to OP and PA, relied in knowing whether AMF could exclusively mobilise more P than the easily available fraction. APs are mostly of sedimentary origin and some igneous reserves also exist in lesser amount (Arif et al. 2017) and the different formation processes may have an impact in their physical, chemical and crystallographic properties and consequently in their effectiveness as a P source (Bolland et al.

1986; Arcand and Schneider 2006). For that reason, it was decided that AP contained in the fungal compartment should be sufficient to detect its P incorporation into the plant tissue.
The water content at the fungal compartments was checked once a week and amended with autoclaved MilliQ water to 19% water volume (field capacity) with the aid of a time-domain reflectometry probe Trime Pico connected to a Trime-FM version P2 (Imko Micromodultechnik GmbH, Ettlingen, Germany).

Plant Phosphorus Content

At each of the five harvesting points, shoots and roots were air dried (70°C, 48 h), weighed, ball milled, and the total P contents were determined. The amount of total P incorporated by the AM plant over time was calculated as the difference of the total P content at each harvest point and the average total P content initially present in the transplanted plants at day 0 (n=5). To determine the P content, an aliquot of shoot and root was incinerated at 480ºC for 8 h, digested with 1 ml 30% HNO_3, filtered through a 0.45 µm syringe filter (PVDF) and analysed by ICP-MS Agilent 7500C (Agilent Technologies Ireland Ltd., Cork, Ireland).

Organic Carbon in the Fungal Compartment

At each sampling point, 2 g from each fungal compartment were air-dried (70°C, 48 h), weighed, and ball milled. Total C was considered as organic carbon (OC), as there was no inorganic C in the fungal compartment. The OC content was determined on an Elementar vario MICRO cube C/N analyser (Elementar GmbH, Hanau, Germany). For the calculation of the total C in the fungal compartment the total carbon content (mg/g) was multiplied by the total weight of substrate in the fungal compartment. For each P source, the mass ratio of C/P was used to evaluate the investment made by the AM plant into its symbiotic partner at the fungal compartment per plant P incorporation (Eq. 1):

$$C/P = \frac{\text{OC (mg per fungal compartment)}}{\text{P incorporated (mg per plant)}} \tag{1}$$

Carbon Dioxide Production in the Fungal Compartment

The CO_2 efflux (mmol CO_2 m^2 h^{-1}) was measured in the fungal compartment with an EGM-4 infrared gas analyser (PP-systems, Hitchin, UK), a close dynamic system (Vermue et al. 2008), during the whole experiment at five days per week. Cumulative CO_2 production was interpolated using a cubic spline function and calculated in mg of CO_2 respired at each fungal compartment for each harvesting day (Gentsch et al. 2018). The cumulative CO_2/P ratio was used as a proxy of the metabolic activity at the fungal compartment per unit P incorporated by the plant (Eq. 2):

$$CO_2/P = \frac{\text{Cumulative } CO_2 \text{ (mg per fungal compartment)}}{\text{P incorporated (mg per plant)}} \quad (2)$$

R. irregularis: Biomass and Energy Storage Estimation

The fatty acid composition of *R. irregularis* DAOM 197198 includes molecules from C16:0 to C22:2 with 16:1ω5 as major fatty acid (Aarle and Olsson 2003; Calonne et al. 2010; Wewer et al. 2014). The PLFA 16:1ω5c is a biomarker to estimate the amount of extraradical AMF mycelia (Olsson and Johansen 2000; Aarle and Olsson 2003; Marschner 2007). The NLFA 16:1ω5c biomarker estimates the amount of energy the fungus allocates for storage and is found in vesicles, spores and extraradical mycelium (Bååth 2003). The NLFA 16:1ω5c is metabolized in the mycelium through the glyoxalate cycle providing the major fungal respiratory substrate (Olsson and Wilhelmsson 2000; Aarle and Olsson 2003; Olsson and Johnson 2005).

Lipids were extracted twice from 16 g of the different fungal compartments using a chloroform-methanol-citrate buffer (1:2:0.8 v/v/v). Extracts were fractionated by solid phase extraction with activated Silica gel (Sigma Aldrich, pore size 60 Å, 70–230 mesh) into neutral lipid fatty acids (NLFA), glycolipids, and phospholipid fatty acids (PLFA) by elution with 5 ml of chloroform, 20 ml of acetone, and 20 ml of methanol, respectively. The PLFA and NLFA samples were exposed to mild alkaline methanolysis, transforming phospholipids and neutral lipids into free fatty acid methyl esters (FAMEs), as described in Frostegård et al. (1991) with modifications by Bischoff et al. (2016). FAMEs were separated by gas chromatography using an Agilent 7890A GC system (Agilent Technologies Ireland Ltd., Cork, Ireland) equipped with a 60 m Zebron capillary GC column (0.25 mm diameter and 0.25 µm film thickness; Phenomenex, Torrance, California, USA) and quantified with a flame ionization detector, using He as carrier gas. Nonadecanoic acid (25 µg) and glyceryl tridodecanoate (25 µg) were used as internal standards during the extraction, and tridecanoic acid methyl ester (15 µg) was added to each sample and standard before analysis as a recovery standard. For the fatty acid identification, the relative retention times were compared to that of the internal standard using the GC ChemStation (B.03.02.341) software (Agilent Technologies Ireland Ltd., Cork, Ireland). In our study, PLFA and NLFA 16:1ω5c were measured to estimate the biomass and energy storage of *R. irregularis* in the fungal compartment, respectively (Johansen et al. 1996; Olsson et al. 1997, 2002; Larsen et al. 1998; Stumpe et al. 2005). Three ratios were calculated to evaluate de C-P trading costs between the AMF and the host plant to acquire the different P sources. The ratios between 16:1ω5c PLFA to plant P uptake (Eq. 3) and NLFA to plant P uptake (Eq. 4) served to estimate the investment made by the tomato plants into their fungal partner in either biomass or energy storage to obtain P from each source, respectively. The ratio between NLFA 16:1ω5c and PLFA 16:1ω5c (Eq. 5) was used as an index for the *R. irregularis* growth strategy of (Green et al. 1999; Rinnan and Bååth 2009). A high NLFA 16:1ω5c/ PLFA 16:1ω5c ratio denotes C allocation to storage products in form of neutral lipids, in order to meet the catabolic needs of the fungus. Additionally, this ratio allows to discriminate between bacterial or fungal origin of the 16:1ω5c fatty acid. When the NLFA 16:1ω5c/PLFA 16:1ω5c>1, the fatty acid is originated from AM fungi and not from bacteria (Olsson 1999; Hammer et al. 2011; Vestberg et al. 2012; Cozzolino et al. 2016).

$$\text{PLFA 16:1}\omega\text{5c}/\text{P} = \frac{\text{PLFA 16:1}\omega\text{5c (total }\mu\text{g per fungal compartment)}}{\text{P incorporated (mg per plant)}} \tag{3}$$

$$\text{NLFA 16:1}\omega\text{5c}/\text{P} = \frac{\text{NLFA 16:1}\omega\text{5c (total }\mu\text{g per fungal compartment)}}{\text{P incorporated (mg per plant)}} \tag{4}$$

$$\text{NLFA}/\text{PLFA} = \frac{\text{NLFA 16:1}\omega\text{5c (total }\mu\text{g per fungal compartment)}}{\text{PLFA 16:1}\omega\text{5c (total }\mu\text{g per fungal compartment)}} \tag{5}$$

Experiment 2

The decision on the sampling day for experiment 2 was made on the basis of the results of Experiment 1. It was decided that sampling point for Experiment 2 would coincide with the moment when all treatments showed the maximum P incorporation from the different P sources. The same growing conditions described in experiment 1 were replicated for two identical groups of plants, which were grown until the date determined at experiment 1. The first group of plants was labelled with ^{13}C stable isotope and the second one was not labelled, for subsequent measurements of the natural abundances. Each identical group contained three biological replications for each P source and a mycorrhized control plant without P. The pulse labelling experiment aimed at determining the amount of freshly assimilated C invested on the one hand, into leaves, shoots, roots, substrates of the plant compartment and on the other one, into the respired CO_2 and the different microbial communities at the fungal compartment.

Photosynthetic Capacity

The photosynthetic capacity of the tomato plants was measured with the LI-6400 (Licor, Lincoln, NE, USA) on recently fully expanded third or fourth leaf from top, one day before the $^{13}CO_2$ pulse labelling. The photosynthetic rate was used to test the effect of the different P sources on the host carbohydrate metabolism, as P deficiency may cause a rapid decrease in photosynthetic capacity (Maathuis 2009). Values were recorded at 22°C in the leaf cuvette, a photon flux density of 1000 µmol/m^2/s, approx. 50% relative humidity, airflow rate of 400 µmol/s, an external CO_2 concentration of 360 ppm and the CO_2 mixer to reference 400 ppm.

^{13}C Pulse Labelling: ^{13}C incorporation into respired CO_2, plant tissues and substrates

Mesocosms were placed in a phyto-chamber (20-22°C, 50–60% relative humidity, photon flux density of 195 µmol/m/s) and connected to the Picarro valve system through a gas inlet and outlet at the fungal compartment to test for air leaks. Synthetic air (CO_2= 415 ppm; $\delta^{13}CO_2 = -11‰$) was pumped through the fungal compartment (4 ml/min) and during the next 120 minutes the stability of the flowrate was checked every ten minutes. Once the absence of leaks in the gas circuit between the mesocosms and the valve system has been verified, twelve sampling bags (three bags per treatment) were connected to the programmable valve system and began filling for the next 120 minutes (Figure 7). After all bags were filled, they were measured sequentially at the Picarro to determine the $^{13}CO_2$ abundance in the respired CO_2 of each mesocosm. As these samples were taken before labelling, the determined values were defined as the background.

Thereafter, all mesocosms were brought to a labelling box (60 x 120 x 50 cm, H x W x D) with forced ventilation (Figure 7) to promote homogeneous $^{13}CO_2$ distribution during the pulse labelling. At the moment of closing the box, the internal CO_2 concentration was 452 ppm ($\delta^{13}CO_2 = -12.23‰$). Another identical set of mesocosms were reserved to determine the natural abundances in the plant tissues and the substrates of both compartments during the course of the experiment. The labelling box was placed inside a greenhouse (20°C, 50–60% relative humidity, photon flux density of 195 µmol/m/s). The $^{13}CO_2$ tracer consisted of 2 g $Ca^{13}CO_3$ (99 atom% ^{13}C) (Sigma-Aldrich, Steinheim, Germany) dissolved in 30 ml H_2SO_4 1M. The labelling box was closed at 10:00 and then the tracer was dissolved in the acid. In each chamber, a glass beaker with 2 g of $Ca^{13}CO_3$ (99 atom% ^{13}C) (Sigma-Aldrich, Steinheim, Germany) was placed.

To dissolve the tracer and release $^{13}CO_2$, 1M H_2SO_4 were added to the glass beaker via a tube fed through the wall of the chamber. At 12:00 h the labelling was stopped by opening the box and moving the mesocosms to another room.

The CO_2 concentration and its ^{13}C signature in the fungal compartment was deterimined by a Picarro G1101-i cavity ring-down spectrometer (Picarro Inc., Santa Clara, CA, USA) (Figure 6 and 7). To allow a higher sampling intensity, a programmable valve system was developed to simultaneously collect a set of samples and then measure them consecutively. Gas samples were collected in 0.6 l gas sampling bags Tedlar® PLV with Thermogreen® LB-2 Septa (Sigma Aldrich). Different pre-tests were carried out to find out the optimum sample volume for the Picarro device to obtain a steady measurement. It was found that at least 300 ml per bag were sufficient for a 10 minutes continuous measurement. The mesocosms were connected to the programmable valve system and the airflow was adjusted to 4 ml/min for each of the twelve mesocosms. Once the airflow through each mesocosm was steady, the first batch of gas bags was connected from 13:00 h until 15:00 h (Figure 7) as described for background sampling, followed by six other sampling points, covering a total of 25.5 h since the end of the $^{13}CO_2$ pulse labelling (Figure 8).

Leaves, shoots, roots, plant and fungal compartment substrates were harvested 27 h after the labelling finished. The samples were freeze-dried, ball milled and aliquots were weighed into tin capsules for isotope ratio mass spectrometry analysis. The content of total C and ^{13}C in the samples was determined with an online continuous flow Elementar vario MICRO cube C/N analyser (Elementar GmbH, Hanau, Germany) coupled to an Isoprime 100 isotope ratio mass spectrometer (Elementar GmbH, Hanau, Germany) using helium (99.999% purity Linde, Germany) as carrier gas. Calibration was carried out with certified solid standards from the International Atomic Energy Agency (IAEA, Vienna, Austria): Sucrose (IAEA-CH-6), cellulose (IAEA-CH-3) and caffeine (IAEA-600) were used for C isotopic composition. Values were reported using international system units (atom $\%^{13}C$). The atomic % of ^{13}C (at$\%^{13}C$) values were calculated with Eq. 6 for all plant, substrate and gas samples,

$$\text{at}\%^{13}\text{C} = \frac{(100 \text{ x AR x } \left(\frac{\delta^{13}\text{C}}{1000+1}\right))}{1+ (\text{AR x } \left(\frac{\delta^{13}\text{C}}{1000+1}\right))} \tag{6}$$

where AR represents the absolute ratio of ^{13}C:^{12}C relative to the Vienna PeeDee Belemnite standard (0.011182) and $\delta^{13}C$ is the value in calibrated ‰ (Slater et al. 2001).

The proportion of freshly assimilated carbon (%C_F) in the different pools (plant tissues, substrates and respired CO_2) was calculated based on a two pool mixing model as given in Eq. 7. Here, at%^{13}C is the ^{13}C enrichment in the labelled (L) or unlabelled (UL) treatment, or the tracer (T), respectively.

$$\%C_F = \left(\frac{\text{at\%}^{13}C_L - \text{at\%}^{13}C_{UL}}{\text{at\%}^{13}C_T - \text{at\%}^{13}C_{UL}}\right) \times 100 \qquad (7)$$

To calculate the content of freshly assimilated C (C_F) in each sample (plant/substrate/gas) the %C_F (calculated with Eq.7) was divided by 100 and the result was multiplied by the total C content of the labelled sample (L) (Eq.8):

$$C_F = \left(\frac{\text{at\%}^{13}C_F}{100}\right) \times C_L \qquad (8)$$

All the freshly assimilated C fractions were summed up and the relative allocation of fresh carbon to the different pools (plant/substrate/gas) was calculated for each treatment.

^{13}C PLFA and ^{13}C NLFA Analysis

Phospholipids and neutral lipids were extracted, saponified to the respective free fatty acids and derivatized following the protocol described for Experiment 1. A set of external standards (multistandard) consisting of 17 fatty acids belonging to different microbial groups (Table 1) and internal standards (Sigma Aldrich, Taufkirchen, Germany) were derivatized and measured together with the PLFA and NLFA fungal compartment samples. The ^{13}C enrichment and the concentration in each fatty acid methyl ester were measured in triplicate with an Agilent 7890A GC system (Agilent Technologies Ireland Ltd., Cork, Ireland) coupled to an Isoprime 100 IR-MS (Elementar GmbH, Hanau, Germany) using helium (99.999% purity Linde, Germany) as gas carrier. The area below each chromatogram peak and the δ^{13}C values of the 17 substances plus internal standards were processed with IonOS 2.0 software (Isoprime, Elementar GmbH, Hanau, Germany) to calculate the concentrations and the at%^{13}C, respectively, of the different PLFA and NLFA biomarkers within the multistandard solution and the samples.

Based on the different amounts of standard substances used (0.15, 0.35, 0.75, 1.5, 3 and 6 µg), we tested for dependency of the δ^{13}C signal on the PLFA amount (Schmitt et al. 2003).

In case both values were correlated, a linear or logarithmic function was fitted and all $\delta^{13}C$ values of samples were corrected for this offset. All samples with chromatogram peak heights lower than 0.1 (nA) were discarded from the analysis as this is the detection limit. All standard substances were also measured in their underivatized form using an Elementar vario MICRO cube elemental analyser (EA) coupled to an Isoprime 100 IRMS. The influence of derivatization C on the $\delta^{13}C$ values of the samples were then corrected by substracting the offset between EA and GC $\delta^{13}C$ values observed for the respective standard substances.

The corrected PLFA and NLFA $\delta^{13}C$ values were calculated to at%^{13}C using Eq. 6. The proportion of freshly assimilated C into each PLFA or NLFA sample ($\%C_{F_PLFA}$ or $\%C_{F\_NLFA}$) was calculated using a two pool mixing model explained in Eq. 7. To calculate the content of freshly assimilated C into PLFA or NLFA biomarkers (C_{PLFA} or C_{PLFA}) of each sample, the $\%C_{F\_PLFA}$ or $\%C_{F\_NLFA}$ (calculated with Eq.7) was divided by 100 and the result was multiplied by the total PLFA or NLFA content of the labelled sample (L) and the total C content within each PLFA or NLFA molecule (Eq.9),

$$C_{PLFA}\ (\mu g\ FC) = \left(\frac{\%C_{F\_PLFA}}{100}\right) \times \left(\frac{M(\mu g\ PLFA\ FC\text{-}C)}{M(\mu g\ PLFA\ FC)} \times \mu g\ PLFA_{L}\right) \qquad (9)$$

where $^{13}C_{PLFA}$ (µg FC) is the freshly assimilated C into PLFA or NLFA biomarkers, M(PLFA-C) is the molar mass of the C in the PLFA or NLFA molecule and M(PLFA) is the molar mass of the PLFA or NLFA molecule. Once the freshly assimilated C contents were obtained in each of the biomarkers for each treatment, they were grouped according to the microbial group to which they belong (Table 1).

Leaf Areas

Total leaf area (cm^2) for each plant was measured with a LI-3100C (Licor, Lincoln, NE, USA) after finishing the pulse chase labelling experiment. Deficiency in P nutrition has been recognized as an important factor reducing the size of the leaf area and consequently limiting light interception and the photosynthetic capacity of leaves (Rodríguez et al. 1998).

Data Analysis

For both experiments, one-way ANOVA analysis of the variance and the Duncan post-hoc test was employed for mean separation ($P<0.05$) between the different P sources offered at the fungal compartment for the different variables at each time point. Data analysis was performed using SPSS v.24 for Windows (IBM Corporation 2016).

Results

Experiment 1

P derived from the three sources was incorporated into the different AM plant, starting from day 35 and lasting until the end of the experiment at day 112. The control M+ did not show any P incorporation (Figure 2). At day 84, all AM plants with access to a P source exhibited the highest P incorporation without a significant difference between the treatments regarding P uptake. At day 112, the inorganic P forms OP and AP facilitated significantly more P for plant growth than for PA. At day 84, the mineral P source AP yielded greater amounts of P than explicable by the pure dissolution of AP and almost 1.6 mg P were found in the AM plant tissues pointing to a potential active mobilisation of the AP by the AMF (Figure 2).
The cumulative CO_2 production (mg) and the organic C content (mg) ratios were significantly larger for those AM plants that had access to AP during the experiment, as compared to OP, PA and the M+ control (Figure 3).

The P incorporated from PA showed larger PLFA and NLFA 16:1ω5c/P ratios than the OP and AP treatments at days 35 and 84 (Figure 4). At day 112, only the PLFA 16:1ω5c/P ratio was significantly larger for AP and PA as compared to the OP. With incubation time, the NLFA 16:1ω5c/PLFA 16:1ω5c ratio increased for all AM plants mobilising a P source in the fungal compartment at days 84 and 112 in contrast to the M+ control (Figure 5).

Experiment 2

Since AM plants with access to a P source exhibited the higher P incorporation and did not exhibited any significant differences in the amount of P incorporated from each source at day 84, a new set of plants was labelled on the same day for comparing the current costs of P acquisition from the different sources.

In case of respired CO_2 derived from freshly assimilated C (µg) measured in the fungal compartment, the M- treatment, without mycorrhiza and without P, showed a non-constant $^{13}CO_2$ respiration over the 25.5 hours pulse-chase experiment. The M- treatment did not show any tendency, contrary to the OP and M+ treatments. Five hours after the pulse labelling (17:00 h) it exhibited an increasing content of ^{13}C in the respired CO_2. The treatment containing AP exhibited significantly higher $^{13}CO_2$ concentration as compared to OP, M+ and M- from the ninth hour (21:00 h) onwards. In the case of PA treatment, there were significant differences to the other treatments after the first three hours (15:00 h) of the pulse-chase experiment. The AP and PA treatments kept a steadily increasing trend regarding the respired $^{13}CO_{2,}$ which lasted until the end of the experiment (Figure 8). Moreover, the sum of $^{13}CO_2$ respired over 25.5 h monitored was significantly highest in case of PA, in comparison to treatments containing OP, AP or M+ (Figure 9).

When analysing the freshly assimilated C into the plant tissues, the highest ^{13}C content in the leaves was found in the AM plants accessing a P source in comparison to the M+ control. AM plants mobilising OP and PA showed the highest ^{13}C content in leaves, followed by AM plants with access to AP in the fungal compartment. Furthermore, the ^{13}C content in the leaves represented around 60% of the total freshly assimilated C in case of AM plants mobilising OP. In addition, AM plants with access to OP exhibited larger leaf areas and photosynthesis rates than the PA, AP or M+ treatments. In the shoot tissues, the significantly highest ^{13}C content was found in the AM plants that accessed PA in comparison to treatments containing OP, AP or M+ (Figure 9). In case of the root samples, AM plants mobilising AP over their mycorrhizal partner exhibited a significantly higher ^{13}C content as compared to those mobilising PA, OP or M+. The ^{13}C content in the plant and fungal compartment substrates derived from freshly assimilated C was significantly higher in all the AM plants that accessed a P source in comparison to the M+ control.

Among the 17 studied PLFA biomarkers, only 11 were labelled (14:0, a15:0, i15:0, 15:0, 16:1w7c, 16:1w5c, 17:0, 18:2w6,9, 18:1w9c, 18:1w7c, 18:0), while in the NLFA samples only the AMF putative biomarker (16:1w5c) showed ^{13}C enrichment. The PLFA AMF putative biomarker contained more than 96% of ^{13}C derived from freshly assimilated C within the PLFA biomarkers for all AM plants accessing a P source, in contrast to the M+ test with only 84% assimilated ^{13}C into the AMF biomarkers. The ^{13}C contents in the AMF biomarkers differed between the treatments, being the ones mobilising PA, AP and OP significantly more abundant compared to the M+ treatment (Figure 10). The results of recently assimilated carbon in the general biomarkers of bacteria (B) were not plotted in Figure 10, as they were up to an order of magnitude lower than the rest of the bacterial markers. The gram-positive (B+) and gram-negative (B-) putative PLFA biomarkers showed a minor percentage of ^{13}C (<0.3% B+; <3.4% B-) derived from freshly assimilated C for all treatments containing a P source when compared to the M+ treatment (7.4% B+; 8.8% B-) (Figure 11). The NLFA AMF putative biomarkers exhibited the highest content of ^{13}C derived from freshly assimilated C for the PA treatment followed by AP and OP in contrast to the M+ control (Figure 12).

Discussion

We tested the ability of *R. irregularis* taking up P from three different sources having a potential different accessibility. To that end, we performed two consecutive experiments, in Experiment 1, we investigated whether exists a C for P trading by the AM plants, when mobilising P derived from the different sources with a potential different accessibility. In Experiment 2, we compared the amount of freshly assimilated C invested by the AM plant into the P acquisition at day 84, when the AM plants exhibited the higher P incorporation and did not exhibited any significant differences in the amount of P incorporated from each source.

Experiment 1

Phosphorus Uptake from Different Accessible Sources by the Arbuscular Mycorrhiza Symbiosis

All P sources were exclusively mobilised by the AMF and incorporated in different amounts into the plant tissues (Figure 2). The AM fungal extraradical mycelium can directly acquire phosphate ions in the form of OP beyond the P depletion zone (Hodge 2016). For its mineralization and phosphate release, the PA needs to be hydrolyzed by phytases of plant or microbial origin (Baldwin et al. 2001). *R. irregularis* DAOM 197198 is able to secrete a type of acid phosphatase, which contributes to the PA mineralization (Tisserant et al. 2012). Several studies have shown the ability of *Rhizophagus* species to hydrolyze organic P and transfer it through the mycelium and making a significant contribution to plant P uptake from sources such as lecithin, RNA, and PA (Tarafdar and Marschner 1994a, b; Joner et al. 2000; Koide and Kabir 2000; Feng et al. 2003; Sato et al. 2015). Utilization of organic P is thus assumed to contribute in a comparable manner to AM plant P nutrition as inorganic P. Likewise, we did not observe significant differences between the amounts of P mobilised from PA and OP during most of the time course experiment. Equal contents of total P (10 mg) where offered in the form of OP and the PA, while for AP the P content was much higher (562 mg), being only 0.09 mg P easily accessible at the fungal compartment. We observed that the AM plant mobilising the AP clearly incorporated more P than the easily available fraction dissolved in water, a fact that points out to an active biological weathering of the primary mineral. At the end of the time course experiment, the treatment containing AP exhibited the significantly highest content of P incorporated in the plant tissues in comparison to all other treatments. It is widely accepted that AM plants can access the P contained in the AP by joint action of roots and hyphae (Murdoch et al. 1967). Souchie et al. (2010) observed a greater clover growth rates in those treatments mycorrhized with AMF and fed with AP as the solely P source in comparison to the non mycorrhized treatments. The AM hyphae appear able to enter AP mineral structures, and without the help of AMF the primary mineral would be too unreactive to benefit most plants (Koele et al. 2014). Burghelea et al.(2015) also demonstrated the importance of AMF enhancing the P uptake in plants by means of mineral weathering. In a very similar approach to ours, they observed over 124 days that AM mycorrhized plants growth was enhanced in presence of rhyolite rocks, due to a P efficient extraction. Different AMF species have demonstrated the ability to mobilise P in AP, e.g. *Glomus fasciculatus* and *G. tenuis* (Powell and Daniel 1978), *G. manihotis* (Ness and Vlek 2000), *G. clarum* (Alloush and Clark 2001) and *G. margarita* (Ramirez et al. 2009). Koele et al. (2014) found mineral weathering 'tunnels' under both arbuscular and ectomycorrhizal associations coupled to P uptake from AP. They pointed out to the rhizosphere acidification as one likely mechanism for the AP weathering by soil mycorrhizal fungi, saprotrophic fungi and bacteria. Two recent papers have also investigated the abilities of *R. irregularis* in phosphate mobilisation from AP (Taktek et al. 2017; Pel et al. 2018). Both publications showed the AMF utilizing poorly soluble AP to deliver phosphate to host plants. Compared to the study of Taktek et al. (2017), we used a complete plant and not a transformed root culture. We also found another significant difference with the investigation of Pel et al. (2018). Contrastingly to our experimental design they did not spatially separate the AP from the roots. Thus, our mesocosms offered an advantageous design, where exclusively the hyphae of *R. irregularis* were able to mobilise the phosphate from OP, PA and the AP. Thus, our results demonstrated the suitability of the double membrane system which prevented the influence of the roots on the P uptake, and therefore evidenced the importance of *R. irregularis* taking up P derived from the different sources.

Metabolic Activity at the Fungal Compartment

We used the respired CO_2, measured at the fungal compartment and normalized for the total OC content as a proxy for the metabolic activity in the fungal compartment. The ratio was significantly higher for treatments containing PA and AP that OP until day 84 (Figure 3). The higher ratios point toward an increased metabolic activity by AMF in order to mobilise P from these sources. Further, the AMF seem to adjust their energy requirements precisely to the accessibility of the P sources, as all C at the fungal compartment was exclusively carried by the fungus during the mobilisation of the different P sources. The CO_2 respiration from the fungal compartments is the sum of the autotrophic and the heterotrophic respiration. The autotrophic respiration comprises the fraction derived from current photosynthates (Olsson et al. 2005). In our case it includes the respiration by mycorrhizal fungi and other microorganisms of the hyphosphere of *R. irregularis*. The heterotrophic respiration is due to the decomposition of OC (Kuzyakov and Gavrichkova 2010) made up mostly of dead hyphae and bacteria, mediated by the microbes present at the fungal compartment. Our results thus show that AMF can energetically adapt its mobilisation processes to a given P source.

Development of *R. Irregularis* under Variable Phosphorus Sources

We used the ratio PLFA and NLFA 16:1ω5c normalized to the total plant P uptake as signature compounds for AMF, since they represent a very small portion of the fatty acids in most other fungi, making them generally useful in the detection of AM mycelium (Van Aarle et al. 2005) (Figure 4). Both ratios showed similar trends, but one order of magnitude higher in the case of the NLFA 16:1ω5c/P, since NLFA 16:1ω5c are generally more abundant in AMF than PLFA 16:1ω5c (Olsson and Wilhelmsson 2000). For the majority of the time course experiment, the PLFA 16:1ω5c/P and NLFA 16:1ω5c/P showed a significant higher value in those treatments containing PA and at day 112 for the treatment with AP, compared to OP and the M+ control. The AM plants mobilising OP and AP only differed when comparing the PLFA 16:1ω5c/P at day 112, indicating a larger hyphae development to mobilise the same amount of P from the primary mineral. One likely justification to increase the fungal infrastructure in the presence of P forms like PA and AP is the need to develop more extraradical hyphae to mobilise and transport P more efficiently to the host plant, as AMF tend to develop more extensively under low nutrient conditions (Bryla and Eissenstat 2005). As would be the case of the PA and AP treatments, which need to be mineralized or weathered before mobilising the phosphate, respectively. Jansa et al (2011) hypothesized on the existence of different mechanisms for P mobilisation by AMF: scavenging of OP, solubilizing AP or hydrolysing PA. They assumed different turnover rates for AMF being associated to the different mobilisation strategies. According to Jansa et al (2011), long-lived hyphae represent an advantage in hardly accessible P sources like AP or PA, as the source needs to be weathered or hydrolyzed once approached. Contrasting to this, they hypothesized short lived hyphae in case of phosphate exploitation in soil, as here a quick exploration of larger soil volumes is key and the process of scavenging OP from soil solution itself does not require comparable amounts of energy.

The NLFA 16:1ω5c/PLFA 16:1ω5c ratio (Figure 5), indicator of preferential C allocation into lipidic storage products (Bååth 2003), was larger for the AM plants mobilising OP and AP at day 56, while for the AM plants mobilising PA the ratio rose at day 84. This fact suggests; first, that AM plants mobilising OP and AP required less energy stored in the form of NLFA; and second, at day 84, when there were not significant differences in the amounts of P incorporated from each source, AMF mobilising PA invested a significantly greater amount of energy in the form of NLFA compared to treatments mobilising OP or AP. Under our experimental conditions, where AM plants took up P exclusively via the mycorrhizal pathway, we observed different trading costs of C for P, depending on the P source. The AM plants mobilising PA exhibited a larger investment into AMF mycelium and metabolism in contrast to the ones mobilising the AP, that showed a smaller investment into the AMF infrastructure but significantly higher metabolism. Our results show different P acquisition strategies that resulted in different C costs for the AM plant. This is in accordance with the hypothesis proposed by Turner (2008), in which the acquisition of the different P compounds are classified by the energy investment that must be made to access the phosphate ions.

Experiment 2

To evaluate the current C-P trading costs made by the AM plants mobilising the different P sources, another set of 84 days old plants were labelled with $^{13}CO_2$, since no significant differences were observed during experiment 1 regarding the amount of P incorporated at day 84. The selected time point offered us a unique opportunity to compare whether the different acquisition strategies described in experiment 1 entailed different energy investments to mobilise the P. For this purpose we used ^{13}C stable isotope labelling in order to trace the pathways of the recent plant assimilated along the trophic chain of AMF and adjacent microbiota.

Photosynthetic Capacity and Leaf Areas

We measured the photosynthetic activity of each AM plant a day before the $^{13}CO_2$ pulse labelling. All AM plants mobilising a P source showed significantly higher photosynthetic capacity than the M+ control, additionally, the AM plants mobilising OP exhibited the highest photosynthetic capacity. As for the photosynthesis rates, also the foliar areas were greater in the AM plants mobilising a P source and plants mobilising OP showed the greatest foliar areas (Figure 9). At least three other studies have reported similar results to ours (Parádi et al. 2003; Wu and Xia 2006; Birhane et al. 2012), where AM plants with bigger leaf areas also showed a higher photosynthetic CO_2 fixation. They attributed to a higher total P content in plant tissues as the main cause for showing an enhanced photosynthetic function, coupled to an enhanced C sink fixation derived into the AMF, in comparison to non AM plants. Our results support the concept that AM plants better mobilising P from the different sources had an impact on the photosynthetic function and the leaf area sizes, to keep a constant photosynthate transfer into the AMF.

Freshly Assimilated Carbon Measured in the Respired CO_2 and The Plant Tissues

The system developed valve system allowed a high resolution of the ^{13}C:^{12}C ratio in CO_2, with 15 samples measured every 2 h, for the first 25.5 h after the $^{13}CO_2$ pulse labelling (Figure 8). The AM plants mobilising PA and AP exhibited significantly higher respired $^{13}CO_2$ compared to OP, M+ and M-, three hours after the pulse labelling (15:00 h) and from the tenth hour (20:30 h) onwards, respectively (Figure 8). We used the respired CO_2 at the fungal compartment as a proxy for the current metabolic activity in the fungal compartment, as AMF derive the recently fixed photosynthates from their host plants, for their growth, respiration, and biological maintenance (Jansa et al. 2011). In nutrient deficient soils, the AMF extraradical mycelium can account as a major source of CO_2 efflux, becoming an important pathway of C flux from host plants to the atmosphere (Nottingham et al. 2010; Tomè et al. 2016). We focused our efforts on accurately registering the first respiration hours in the fungal compartment, as Grimoldi et al. (2006), Moyano et al. (2007) and Bahn et al. (2009) demonstrated that AM mycelia may provide a rapid and important pathway of C flux from plants to the soil and atmosphere, providing evidence of direct respiratory losses by AMF. Johnson et al. (2002) also highlighted the value of thorough surveys to track short term C transfer dynamics of AM plants to soil, as many pulse labelling trials have used relatively long fixation periods or have collected samples many hours after the end of labelling and consequently may have overlooked the maximum C yields from roots to AMF. Unlike experiment 1, in experiment 2 only autotrophic respiration was determined at the fungal compartment. However, as we observed in experiment 1 (Figure 3), the results of experiment 2 also exhibited higher metabolic rates in those AM plants mobilising PA and AP than taking up OP (Figure 8). In line with Bahn et al. (2009), we detected that the new photoassimilates were transported and respired belowground within the first 3 h after the pulse labelling started, for the AM plants mobilising PA. And similar to our results, Johnson et al. (2002) showed that the release of $^{13}CO_2$ from AMF peaked 9–14 h after labelling and during the first 21 h. Most of the C lost by the plants was respired by the AMF and mostly declined after the first 24 h. At the end of the respiration monitoring in the fungal compartment, AM plants mobilising P from the PA showed the significantly highest cumulative $^{13}CO_2$, (Figure 9), pointing to a greater C investment to mobilise the equal P amounts, in contrast to the other treatments

Twenty seven hours after the ^{13}C pulse labelling, we found a different ^{13}C partitioning into the plant tissues (Figure 9). The ^{13}C contents in the leaves, shoots, roots and substrates from plant and fungal compartments were significantly higher for treatments mobilising OP, PA and AP *versus* the M+ treatment. As Johnson (2010) theorized in her trade balance model, AM plants might partition more C below ground under P limitation conditions to develop the capacity for soil P extraction. The model predicts that the function of AM symbioses depends on the stoichiometry of available N and P. There will be mutualistic benefits at no N limitation and low P availability because a non-limited N supply tends to increase the photosynthetic capacity of the host plant. In our experimental design, the N was not limited since it was applied in the nutrient solution weekly. Our results corroborate Johnson's postulates, since all AM plants that mobilised and incorporated a P source exhibited higher photosynthetic capacity foliar areas, and ^{13}C contents in all the plant tissue compartments compared to the M+ treatment (Figure 9), to supply more C to the AMF for mining the P sources with different accessibility.

Freshly Assimilated Carbon Assimilated in the Different Microbial Communities

We traced the fate of the freshly assimilated C into the microbial communities present at the different fungal compartments with the aid of fatty acid biomarkers. All AM plants with access to PA mobilised significantly higher total contents of freshly assimilated C into their AMF PLFA biomarkers (Figure 10). When analysing the results of the relative content of freshly assimilated C (%) to their fungal counterparts, > 96% of the freshly assimilated C within the PLFA biomarkers were found in AMF PLFA biomarkers mobilising a P source, in contrast to the M+ control, where 87% of the PLFAs were assigned to fungi. It is worth noting that no significant differences were found in the absolute values of assimilated ^{13}C into any of the B+ and B- bacterial biomarkers (Figure 11), but treatments containing PA and AP showed the significant lowest relative values of B- bacterial biomarkers (<1%) *versus* OP and M+. Our results revealed that treatments mobilising a P source, mostly showed the highest ^{13}C content in the AMF hyphae along with the significantly lowest ^{13}C values in the bacterial communities. This finding highlights that at day 84, when no difference in the amount of P incorporated in the AM plant tissues was found, the majority of the C assimilated by the AM plant remained within the fungal structures and that a small fraction was delivered to AMF accompanying microbiota, since we inoculated the tomato plants with the strain of *R. irregularis* DOAM 197198 not grown in a monoxenic culture, thus carrying the microorganisms naturally associated with its hyphosphere. Zhang et al. (2016) pointed out that the AMF interactions with specific bacteria depend upon background P availability. The AMF may act as a rapid hub for recent photosynthates providing important niches to hyphae-associated soil bacterial communities which are generally composed of specific assemblages of species that differ from those in the bulk soil (Toljander et al. 2007; Kaiser et al. 2015; Bandyopadhyay et al. 2017). We consider the possibility of greater relevance for the bacterial communities in the P acquisition, as the hyphae of AMF may provide an increased area for interactions with other soil microorganisms, especially bacteria which may in turn synergistically interact with AMF and thereby promote plant growth (Taktek et al. 2015). Among the most effective bacterial phosphate mobilisers are species of the B+ genera *Streptomyces*, *Kocuria, Arthrobacter*, *Nocardiodes*, *Microbacterium*, *Bacillus* and the B- genera *Pseudomonas, Enterobacter, Burkholderia, Serratia, Citrobacter, Xanthomonas, Rhizobium, Azospirillum, Klebsiella* (Battini et al. 2016; Osorio et al. 2017). The mechanisms to access the more stable P forms include phosphatase synthesis (E.g. acid phosphatase, phytase), soil pH lowering and/or ligand exchange reactions mediated by low molecular weight organic acids (Souchie et al. 2010; Osorio et al. 2017). Although not significantly different from the M+ control, treatments with PA and AP in their fungal compartments displayed the highest absolute ^{13}C contents in B- bacterial biomarkers 27 hours after $^{13}CO_2$ labelling (Figure 11). The results would show AMF altering the diversity and structure of bacterial communities in the fungal compartment and fuelling P solubilization (AP) or hydrolyzation (PA). In this regard, Kim et al. (1998) and Taktek et al. (2015, 2017) suggested a synergistic interaction between the hyphobacteria able to solubilize insoluble phosphate and an AMF to mobilise P from AP. A similar reasoning was made by Selvakumar et al. (2016), Lecomte et al. (2011) and Battini et al. (2016), who showed a synergistic interaction between the AMF and some bacteria with abilities to mineralize the PA. We estimated the cost of establishing an AM by means of the NLFA 16:1ω5c biomarker (Bååth 2003), since the AM pathway seems to respond quickly to changing soil nutrient by adapting the amount of plant C channelled through the fungus (Kaiser et al. 2015). The NLFA 16:1ω5c is stored in intraradical vesicles, spores, extraradical mycelium and metabolized in the mycelium through the glyoxalate cycle, providing the major fungal energy source as respiratory substrate (Olsson and Wilhelmsson 2000; Aarle and Olsson 2003; Olsson and Johnson 2005). The freshly assimilated C incorporated into AMF NLFA biomarkers clearly indicated that PA was the P source with the most expensive acquisition cost, followed by AP and OP in contrast to the M+ control (Figure 12). The results reveal that AM plants with access to PA and AP diversified the fate of recently assimilated C, incorporating it into the PLFA and NLFA 16:1ω5c. The more stable and therefore less accessible P sources PA and AP received greater amounts of photosynthates that were invested in further growth (PLFA 16:1ω5c) and energy accumulation (NLFA 16:1ω5c) along with a potential contribution of B- bacteria in the mobilisation of phosphate from both P sources.

Conclusions

In this study we showed that AM plants took up P derived from the three P sources OP, AP, and PA offered at the fungal compartment, exclusively via the mycorrhizal pathway with different C-P trading costs. We identified different P acquisition strategies to mobilise the less available P sources PA and AP. In the fungal compartment, both sources exhibited the highest investments into fungal infrastructure (PLFA) and also the highest metabolic rates (respired CO_2). We also observed that B- bacteria likely contribute to P acquisition from PA and AP, suggesting a synergistic cooperation may occur between both organisms. Our results point towards the mobilisation of the less available P sources required longer lived mycelium infrastructure with higher metabolic rates, in contrast to the OP with a faster hyphal turnover and lower requirements of freshly assimilated C.

The C investments into P mobilisation from P sources with differing accessibilities suggests the existence of a nexus between AMF P mining strategies and the amounts of C accumulated in terrestrial soils.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

We want to thank the German Federal Ministry of Education and Research for the funding of this project in the framework of the DFG-RTG 1798 “Signaling at the Plant-Soil Interface”.

Acknowledgments

The authors are thankful for the great help and guidance received by Norman Gentsch, Silke Bokeloh, Elke Eichmann-Prusch, Ulrike Pieper, Heike Steffen, and Michael Klatt.

References

Aarle IM Van, Olsson PA (2003) Fungal Lipid Accumulation and Development of Mycelial Structures by Two Arbuscular Mycorrhizal Fungi Fungal Lipid Accumulation and

Development of Mycelial Structures by Two Arbuscular Mycorrhizal Fungi. Appl Environ Microbiol 69:6762–6767. doi: 10.1128/AEM.69.11.6762

Alloush GA, Clark RB (2001) Maize response to phosphate rock and arbuscular mycorrhizal fungi in acidic soil. Commun Soil Sci Plant Anal 32:231–254. doi: 10.1081/CSS-100103004

Arcand MM, Schneider KD (2006) Plant- And microbial-based mechanisms to improve the agronomic effectiveness of phosphate rock: A review. An Acad Bras Cienc 78:791–807. doi: 10.1590/S0001-37652006000400013

Arif MS, Shahzad SM, Yasmeen T, et al (2017) Improving Plant Phosphorus (P) Acquisition by Phosphate-Solubilizing Bacteria. In: Essential Plant Nutrients. Springer International Publishing, Cham, pp 513–556

Bååth E (2003) The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. Microb Ecol 45:373–383. doi: 10.1007/s00248-003-2002-y

Bahn M, Schmitt M, Siegwolf R, et al (2009) Does Photosynthesis Affect Grassland Soil-Respired CO_2 and Its Carbon Isotope Composition on a Diurnal Timescale? New Phytol 182:451–460. doi: 10.2307/30225853

Baldwin JC, Karthikeyan AS, Raghothama KG (2001) LEPS2, a phosphorus starvation-induced novel acid phosphatase from tomato. Plant Physiol 125:728–37. doi: 10.1104/pp.125.2.728

Bandyopadhyay P, Bhuyan SK, Yadava PK, et al (2017) Emergence of plant and rhizospheric microbiota as stable interactomes. Protoplasma 254:617–626

Battini F, Cristani C, Giovannetti M, Agnolucci M (2016) Multifunctionality and diversity of culturable bacterial communities strictly associated with spores of the plant beneficial symbiont Rhizophagus intraradices. Microbiol Res 183:68–79. doi: 10.1016/j.micres.2015.11.012

Birhane E, Sterck FJ, Fetene M, et al (2012) Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. Oecologia 169:895–904. doi: 10.1007/s00442-012-2258-3

Bischoff N, Mikutta R, Shibistova O, et al (2016) Land-use change under different climatic conditions: Consequences for organic matter and microbial communities in Siberian steppe soils. Agric Ecosyst Environ 235:253–264. doi: 10.1016/j.agee.2016.10.022

Bolland MDA, Weatherley AJ, Gilkes RJ, Bowden JW (1986) Granular reactive apatite rock phosphate is not an effective phosphorus fertilizer in the short term on lateritic soils in south-western Australia. Aust J Exp Agric 26:217–225. doi: 10.1071/EA9860217

Brundrett M, Bougher N, Dell B, et al (1996) Working with mycorrhizas in forestry and agriculture. Australian Centre for International Agricultural Research, Canberra

Bryla DR, Eissenstat DM (2005) Respiratory Costs of Mycorrhizal Associations. In: Plant Respiration. Springer-Verlag, Berlin/Heidelberg, pp 207–219

Burghelea C, Zaharescu DG, Dontsova K, et al (2015) Mineral nutrient mobilisation by plants from rock: influence of rock type and arbuscular mycorrhiza. Biogeochemistry 124:187–203. doi: 10.1007/s10533-015-0092-5

Calonne M, Fontaine J, Debiane D, et al (2010) Propiconazole Toxicity on the Non-Target Organism , the Arbuscular Mycorrhizal Fungus, Glomus irregulare. Fungicides 325–346

Cozzolino V, Di Meo V, Monda H, et al (2016) The molecular characteristics of compost affect plant growth, arbuscular mycorrhizal fungi, and soil microbial community composition. Biol Fertil Soils 52:15–29. doi: 10.1007/s00374-015-1046-8

Daubois L, Beaudet D, Hijri M, De La Providencia I (2016) Independent mitochondrial and nuclear exchanges arising in Rhizophagus irregularis crossed-isolates support the presence of a mitochondrial segregation mechanism Ecological and evolutionary microbiology. BMC Microbiol 16:11. doi: 10.1186/s12866-016-0627-5

Douds DD, Johnson NC (2007) Contributions of arbuscular mycorrhizas to soil biological fertility. In: Soil Biological Fertility: A Key to Sustainable Land Use in Agriculture. Springer Netherlands, Dordrecht, pp 129–162

Feng G, Song Y. C, Li X. L, Christie P (2003) Contribution of arbuscular mycorrhizal fungi to utilization of organic sources of phosphorus by red clover in a calcareous soil. Appl Soil Ecol 22:139–148. doi: 10.1016/S0929-1393(02)00133-6

Fitter AH, Graves JD, Watkins NK, et al (1998) Carbon transfer between plants and its control in networks of arbuscular mycorrhizas. Funct Ecol 12:406–412. doi: 10.1046/j.1365-2435.1998.00206.x

Frostegård Å, Tunlid A, Bååth E (1991) Microbial biomass measured as total lipid phosphate in soils of different organic content. J Microbiol Methods 14:151–163. doi: 10.1016/0167-7012(91)90018-L

Gentsch N, Wild B, Mikutta R, et al (2018) Temperature response of permafrost soil carbon is attenuated by mineral protection. Glob Chang Biol 24:3401–3415. doi: 10.1111/gcb.14316

Green H, Larsen J, Olsson PA, et al (1999) Suppression of the biocontrol agent Trichoderma harzianum by mycelium of the arbuscular mycorrhizal fungus Glomus intraradices in root-free soil. Appl Environ Microbiol 65:1428–1434

Grimoldi AA, Kavanová M, Lattanzi FA, et al (2006) Arbuscular mycorrhizal colonization on carbon economy in perennial ryegrass: Quantification by 13CO2/12CO 2 steady-state labelling and gas exchange. New Phytol 172:544–553. doi: 10.1111/j.1469-8137.2006.01853.x

Hammer EC, Nasr H, Pallon J, et al (2011) Elemental composition of arbuscular mycorrhizal fungi at high salinity. Mycorrhiza 21:117–129. doi: 10.1007/s00572-010-0316-4

Hewitt EJ (1966) Sand and water culture methods used in the study of plant nutrition, Technichal. University of Bristol Agricultural and Horticultural Research Station, Long Ashton, Bristol., East Malling , Maidstone, Kent.

Hodge A (2016) Accessibility of Inorganic and Organic Nutrients for Mycorrhizas. In: Mycorrhizal Mediation of Soil: Fertility, Structure, and Carbon Storage. Elsevier, pp 129–148

IBM Corporation (2016) IBM SPSS Statistics for Windows, Version 24.0. 2016

Jansa J, Finlay RD, Wallander H, et al (2011) Role of Mycorrhizal Symbioses in Phosphorus Cycling. In: Phosphorus in Action. Biological Processes in Soil Phosphorus Cycling. pp 137–168

Johansen A, Finlay RD, Olsson PA (1996) Nitrogen metabolism of external hyphae of the arbuscular mycorrhizal fungus Glomus intraradices. New Phytol 133:705–712. doi: 10.1111/j.1469-8137.1996.tb01939.x

Johnson D, Leake JR, Ostle N, et al (2002) In situ 13CO2 pulse-labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. New Phytol 153:327–334. doi: 10.1046/j.0028-646X.2001.00316.x

Johnson NC (2010) Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. New Phytol 185:631–647. doi: 10.1111/j.1469-8137.2009.03110.x

Joner EJ, Van Aarle IM, Vosatka M (2000) Phosphatase activity of extra-radical arbuscular mycorrhizal hyphae: A review. In: Plant and Soil. pp 199–210

Kahiluoto H, Vestberg M (1998) The Effect of Arbuscular Mycorrhiza on Biomass Production and Phosphorus Uptake from Sparingly Soluble Sources By Leek (Allium porrum L.) in Finnish Field Soils. Biol Agric Hortic 16:65–85. doi: 10.1080/01448765.1998.9755219

Kaiser C, Kilburn MR, Clode PL, et al (2015) Exploring the transfer of recent plant photosynthates to soil microbes: Mycorrhizal pathway vs direct root exudation. New Phytol 205:1537–1551. doi: 10.1111/nph.13138

Khosravi A, Zarei M, Ronaghi A (2017) Influence of Biofertilizers and Phosphate Sources on the Phosphorus Uptake of Lettuce and Chemical Forms of Phosphorus in Soil. Commun Soil Sci Plant Anal 1–14. doi: 10.1080/00103624.2017.1416396

Kim Y, Jordan D, McDonald GA (1998) Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biol Fertil Soils 26:79–87. doi: 10.1007/s003740050347

Koele N, Dickie IA, Blum JD, et al (2014) Ecological significance of mineral weathering in ectomycorrhizal and arbuscular mycorrhizal ecosystems from a field-based comparison. Soil Biol Biochem 69:63–70. doi: 10.1016/j.soilbio.2013.10.041

Köhl L, Lukasiewicz CE, Van der Heijden MGA (2016) Establishment and effectiveness of inoculated arbuscular mycorrhizal fungi in agricultural soils. Plant Cell Environ 39:136–146. doi: 10.1111/pce.12600

Koide RT, Kabir Z (2000) Extraradical hyphae of the mycorrhizal fungus Glomus intraradices can hydrolyse inorganic phosphate. New Phytol 148:511–517. doi: 10.1046/j.1469-8137.2000.00776.x

Kuzyakov Y, Gavrichkova O (2010) Time lag between photosynthesis and carbon dioxide efflux from soil: A review of mechanisms and controls. Glob Chang Biol 16:3386–3406. doi: 10.1111/j.1365-2486.2010.02179.x

Lambers H, Mougel C, Jaillard B, Hinsinger P (2009) Plant-microbe-soil interactions in the rhizosphere: an evolutionary perspective. Plant Soil 321:83–115. doi: 10.1007/s11104-009-0042-x

Larsen J, Olsson PA, Jakobsen I (1998) The use of fatty acid signatures to study mycelial interactions between the arbuscular mycorrhizal fungus Glomus intraradices and the saprotrophic fungus Fusarium culmorum in root-free soil. Mycol Res 102:1491–1496. doi: 10.1017/S0953756298006558

Leake JR, Read DJ (2016) Mycorrhizal Symbioses and Pedogenesis Throughout Earth's History. In: Mycorrhizal Mediation of Soil: Fertility, Structure, and Carbon Storage. Elsevier, pp 9–33

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 317:43–51. doi: 10.1111/j.1574-6968.2011.02209.x

Li C, Kuyper TW, van der Werf W, et al (2018) Testing for complementarity in phosphorus resource use by mixtures of crop species. Plant Soil 1–15. doi: 10.1007/s11104-018-3732-4

Maathuis FJ (2009) Physiological functions of mineral macronutrients. Curr Opin Plant Biol 12:250–258. doi: 10.1016/J.PBI.2009.04.003

Mäder P, Vierheilig H, Alt M, Wiemken A (1993) Boundaries between soil compartments formed by microporous hydrophobic membranes (GORE-TEXR) can be crossed by vesicular-arbuscular mycorrizal fungi but not by ions in the soil solution. Plant Soil 152:201–206. doi: 10.1007/BF00029089

Mäder P, Vierheilig H, Streitwolf-Engel R, et al (2000) Transport of 15N from a soil compartment separated by a polytetrafluoroethylene membrane to plant roots via the hyphae of arbuscular mycorrhizal fungi. New Phytol 146:155–161. doi: 10.1046/j.1469-8137.2000.00615.x

Marschner P (2007) Soil Microbial Community Structure and Function Assessed by FAME, PLFA and DGGE — Advantages and Limitations. In: Advanced Techniques in Soil Microbiology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp 181–200

Moyano FE, Kutsch WL, Schulze ED (2007) Response of mycorrhizal, rhizosphere and soil basal respiration to temperature and photosynthesis in a barley field. Soil Biol Biochem 39:843–853. doi: 10.1016/j.soilbio.2006.10.001

Murdoch CL, Jackobs JA, Gerdemann JW (1967) Utilization of phosphorus sources of different availability by mycorrhizal and non-mycorrhizal maize. Plant Soil 27:329–334. doi: 10.1007/BF01376326

Ness RLLL, Vlek PLGG (2000) Mechanism of calcium and phosphate release from hydroxy-apatite by mycorrhizal hyphae. Soil Sci Soc Am J 64:949–955. doi: 10.2136/sssaj2000.643949x

Nottingham AT, Turner BL, Winter K, et al (2010) Arbuscular mycorrhizal mycelial respiration in a moist tropical forest. New Phytol 186:957–967. doi: 10.1111/j.1469-8137.2010.03226.x

Olsson P, Linder S, Giesler R, Högberg P (2005) Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. Glob Chang Biol 11:1745–1753. doi: 10.1111/j.1365-2486.2005.001033.x

Olsson PA (1999) Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. FEMS Microbiol Ecol 29:303–310. doi: 10.1016/S0168-6496(99)00021-5

Olsson PA, Bååth E, Jakobsen I (1997) Phosphorus effects on the mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by analysis of fatty acid signatures. Appl Environ Microbiol 63:3531–3538

Olsson PA, Johansen A (2000) Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. Mycol Res 104:429–434. doi: 10.1017/S0953756299001410

Olsson PA, Johnson NC (2005) Tracking carbon from the atmosphere to the rhizosphere. Ecol Lett 8:1264–1270. doi: 10.1111/j.1461-0248.2005.00831.x

Olsson PA, van Aarle IM, Allaway WG, et al (2002) Phosphorus Effects on Metabolic Processes in Monoxenic Arbuscular Mycorrhiza Cultures. Plant Physiol 130:1162–1171. doi: 10.1104/pp.009639

Olsson PA, Wilhelmsson P (2000) The growth of external AM fungal mycelium in sand dunes and in experimental systems. Plant Soil 226:161–169. doi: 10.1023/A:1026565314345

Osorio NW, Osorno L, Leon JD, Álvarez C (2017) Plant-Microbe Interactions for Phosphate Management in Tropical Soils. In: Essential Plant Nutrients. Springer International Publishing, Cham, pp 491–512

Parádi I, Bratek Z, Láng F (2003) Influence of arbuscular mycorrhiza and phosphorus supply on polyamine content, growth and photosynthesis of Plantago lanceolata. Biol Plant 46:563–569. doi: 10.1023/A:1024819729317

Parniske M (2008) Arbuscular mycorrhiza: the mother of plant root endosymbioses. Nat Rev Microbiol 6:763–75. doi: 10.1038/nrmicro1987

Pel R, Dupin S, Schat H, et al (2018) Growth benefits provided by different arbuscular mycorrhizal fungi to Plantago lanceolata depend on the form of available phosphorus. Eur J Soil Biol 88:89–96. doi: 10.1016/j.ejsobi.2018.07.004

Powell CL, Daniel J (1978) Mycorrhizal fungi stimulate uptake of soluble and insoluble phosphate fertilizer from a phosphate-deficient soil. New Phytol 80:351–358. doi: 10.1111/j.1469-8137.1978.tb01568.x

Ramirez R, Mendoza B, Lizaso JI (2009) Mycorrhiza effect on maize P uptake from phosphate rock and superphosphate. Commun Soil Sci Plant Anal 40:2058–2071. doi: 10.1080/00103620902960583

Raven JA, Lambers H, Smith SE, Westoby M (2018) Costs of acquiring phosphorus by vascular land plants: patterns and implications for plant coexistence. New Phytol. 217:1420–1427

Řezáčová V, Slavíková R, Konvalinková T, et al (2017) Imbalanced carbon-for-phosphorus exchange between European arbuscular mycorrhizal fungi and non-native Panicum grasses—A case of dysfunctional symbiosis. Pedobiologia (Jena) 62:48–55. doi: 10.1016/j.pedobi.2017.05.004

Rinnan R, Bååth E (2009) Differential utilization of carbon substrates by bacteria and fungi in tundra soil. Appl Environ Microbiol 75:3611–3620. doi: 10.1128/AEM.02865-08

Rodríguez D, Keltjens WG, Goudriaan J (1998) Plant leaf area expansion and assimilate production in wheat (Triticum aestivum L.) growing under low phosphorus conditions. Plant Soil 200:227–240. doi: 10.1023/A:1004310217694

Rosling A, Lindahl BD, Finlay RD (2004) Carbon allocation to ectomycorrhizal roots and mycelium colonising different mineral substrates. New Phytol 162:795–802. doi:

10.1111/j.1469-8137.2004.01080.x

Ryan MH, Tibbett M, Edmonds-Tibbett T, et al (2012) Carbon trading for phosphorus gain: The balance between rhizosphere carboxylates and arbuscular mycorrhizal symbiosis in plant phosphorus acquisition. Plant, Cell Environ 35:2170–2180. doi: 10.1111/j.1365-3040.2012.02547.x

Sato T, Ezawa T, Cheng W, Tawaraya K (2015) Release of acid phosphatase from extraradical hyphae of arbuscular mycorrhizal fungus Rhizophagus clarus. Soil Sci Plant Nutr 61:269–274. doi: 10.1080/00380768.2014.993298

Schmitt J, Glaser B, Zech W (2003) Amount-dependent isotopic fractionation during compound-specific isotope analysis. Rapid Commun Mass Spectrom 17:970–977. doi: 10.1002/rcm.1009

Selvakumar G, Krishnamoorthy R, Kim K, Sa TM (2016) Genetic diversity and association characters of bacteria isolated from arbuscular mycorrhizal fungal spore walls. PLoS One 11:1–16. doi: 10.1371/journal.pone.0160356

Shen J, Yuan L, Zhang J, et al (2011) Phosphorus Dynamics: From Soil to Plant. Plant Physiol 156:997–1005. doi: 10.1104/pp.111.175232

Slater C, Preston T, Weaver LT (2001) Stable isotopes and the international system of units. Rapid Commun Mass Spectrom 15:1270–1273. doi: 10.1002/rcm.328

Smith SE, Read DJ (2008) Mycorrhizal symbiosis. Academic Press

Smits MM, Bonneville S, Benning LG, et al (2012) Plant-driven weathering of apatite - the role of an ectomycorrhizal fungus. Geobiology 10:445–456. doi: 10.1111/j.1472-4669.2012.00331.x

Smits MM, Bonneville S, Haward S, Leake JR (2008) Ectomycorrhizal weathering, a matter of scale? Mineral Mag 72:131–134. doi: 10.1180/minmag.2008.072.1.131

Souchie EL, Azcón R, Barea JM, et al (2010) Enhancement of clover growth by inoculation of P-solubilizing fungi and arbuscular mycorrhizal fungi. An Acad Bras Cienc 82:771–777. doi: 10.1590/S0001-37652010000300023

Steidinger BS, Turner BL, Corrales A, Dalling JW (2015) Variability in potential to exploit different soil organic phosphorus compounds among tropical montane tree species. Funct

Ecol 29:121–130. doi: 10.1111/1365-2435.12325

Stumpe M, Carsjens J-GG, Stenzel I, et al (2005) Lipid metabolism in arbuscular mycorrhizal roots of Medicago truncatula. Phytochemistry 66:781–791. doi: 10.1016/j.phytochem.2005.01.020

Taktek S, St-Arnaud M, Piché Y, et al (2017) Igneous phosphate rock solubilization by biofilm-forming mycorrhizobacteria and hyphobacteria associated with Rhizoglomus irregulare DAOM 197198. Mycorrhiza 27:13–22. doi: 10.1007/s00572-016-0726-z

Taktek S, Trépanier M, Servin PM, et al (2015) Trapping of phosphate solubilizing bacteria on hyphae of the arbuscular mycorrhizal fungus Rhizophagus irregularis DAOM 197198. Soil Biol Biochem 90:1–9. doi: 10.1016/j.soilbio.2015.07.016

Tarafdar JC, Marschner H (1994a) Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. Soil Biol Biochem 26:387–395. doi: 10.1016/0038-0717(94)90288-7

Tarafdar JC, Marschner H (1994b) Efficiency of VAM hyphae in utilisation of organic phosphorus by wheat plants. Soil Sci Plant Nutr 40:593–600. doi: 10.1080/00380768.1994.10414298

Tarafdar JC, Yadav RS, Niwas R (2002) Relative efficiency of fungal intra- and extracellular phosphatases and phytase. J Plant Nutr Soil Sci 165:17–19. doi: 10.1002/1522-2624(200202)165:1<17::AID-JPLN17>3.0.CO;2-C

Tisserant E, Kohler A, Dozolme-Seddas P, et al (2012) The transcriptome of the arbuscular mycorrhizal fungus Glomus intraradices (DAOM 197198) reveals functional tradeoffs in an obligate symbiont. New Phytol 193:755–769. doi: 10.1111/j.1469-8137.2011.03948.x

Toljander JF, Lindahl BDBD, Paul LR, et al (2007) Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. FEMS Microbiol Ecol 61:295–304. doi: 10.1111/j.1574-6941.2007.00337.x

Tomè E, Ventura M, Folegot S, et al (2016) Mycorrhizal contribution to soil respiration in an apple orchard. Appl Soil Ecol 101:165–173. doi: 10.1016/j.apsoil.2016.01.016

Turner BL (2008) Resource partitioning for soil phosphorus: A hypothesis. J Ecol 96:698–702. doi: 10.1111/j.1365-2745.2008.01384.x

Van Aarle IM, Cavagnaro TR, Smith SE, et al (2005) Metabolic activity of Glomus intraradices in Arum- and Paris-type arbuscular mycorrhizal colonization. New Phytol 166:611–618. doi: 10.1111/j.1469-8137.2005.01340.x

Vermue E, Elbers J, Hoosbeek M (2008) A Comparative Field Study of Four Soil Respiration Systems

Vestberg M, Palojarvi A, Pitkanen T, et al (2012) Neutral lipid fatty acid analysis is a sensitive marker for quantitative estimation of arbuscular mycorrhizal fungi in agricultural soil with crops of different mycrotrophy. Agric Food Sci 21:12–27

Vierheilig H, Alt-Hug M, Engel-Streitwolf R, et al (1998a) Studies on the attractional effect of root exudates on hyphal growth of an arbuscular mycorrhizal fungus in a soil compartment-membrane system. Plant Soil 203:137–144. doi: 10.1023/A:1004329919005

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

Walker TW, Syers JK (1976) The fate of phosphorus during pedogenesis. Geoderma 15:1–19. doi: 10.1016/0016-7061(76)90066-5

Watkins NK, Fitter AH, Graves JD, Robinson D (1996) Carbon transfer between C3 and C4 plants linked by a common mycorrhizal network, quantified using stable carbon isotopes. Soil Biol Biochem 28:471–477. doi: http://dx.doi.org/10.1016/0038-0717(95)00189-1

Wewer V, Brands M, Dörmann P (2014) Fatty acid synthesis and lipid metabolism in the obligate biotrophic fungus Rhizophagus irregularis during mycorrhization of Lotus japonicus. Plant J 79:398–412. doi: 10.1111/tpj.12566

Wu Q-S, Xia R-X (2006) Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. J Plant Physiol 163:417–425. doi: 10.1016/j.jplph.2005.04.024

Zhang L, Xu M, Liu Y, et al (2016) Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. New Phytol 210:1022–1032. doi: 10.1111/nph.13838

Table 1. List of analysed phospholipid fatty acid biomarkers and their associated microbial group.

Fatty Acid Biomarker	Microbial groups (Code)	References
16:1ω5c	Fungal markers present in AMF (F)	(Olsson et al. 1995, 1997, 2002; Johansen et al. 1996; Larsen et al. 1998; Green et al. 1999; Olsson and Wilhelmsson 2000; Madan et al. 2002; Aarle and Olsson 2003; Stumpe et al. 2005)
18:1ω7c		
18:1ω9c		
18:2ω6,9		
14:0	General bacterial marker (B)	(Willers et al. 2015)
15:0		
17:0		
18:0		
16:1ω7c	Gram-negative bacteria (B-)	
cy19:0		
a15:0	Gram-positive bacteria (B+)	
a17:0		
i15:0		
i16:0		
i17:0		
20:4ω6c	Protozoa and nematodes (PN)	
10Me16:1	Actinomycetes (A)	

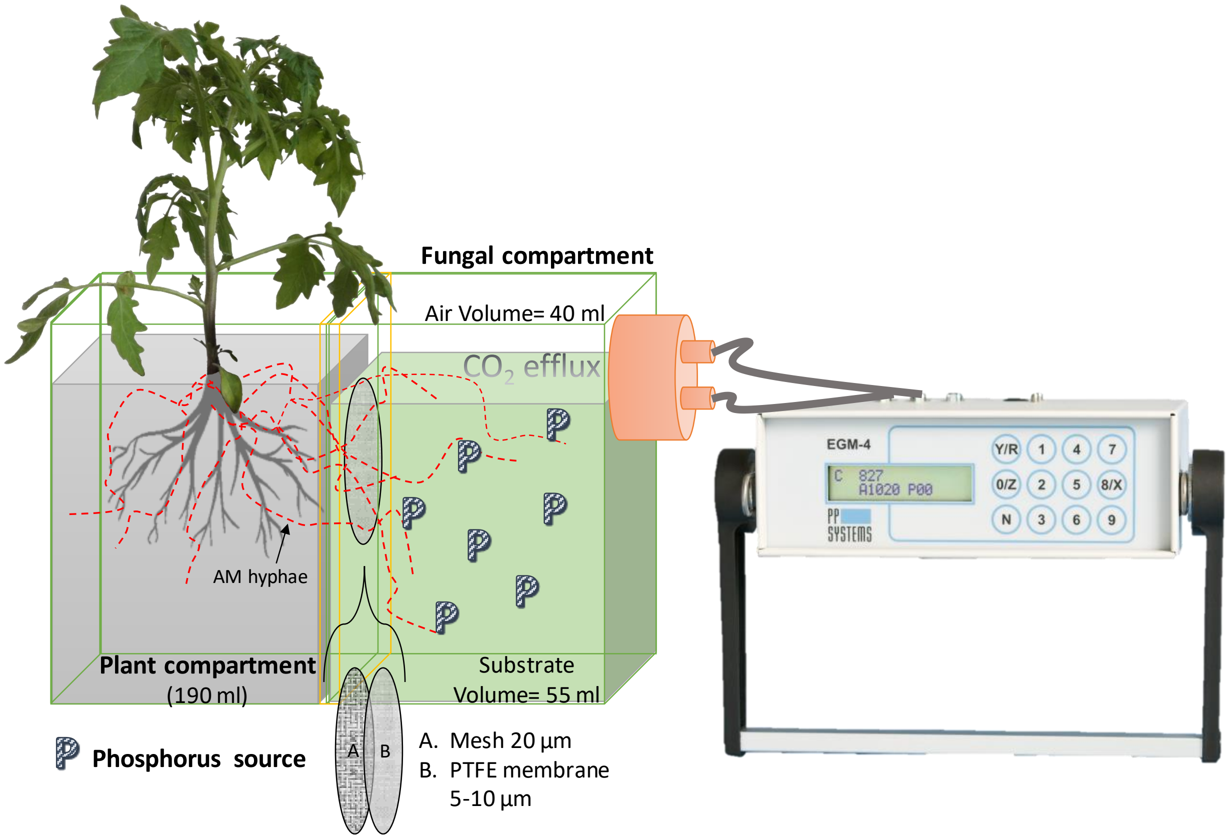


Figure 1. Mesocosms scheme containing an AM mycorrhizal plant in the left compartment and a fungal compartment in the right compartment, containing one of the different P forms orthophosphate in solution as readily available P form, apatite as mineral P source, phytate as organic P source or a blank with no P. The plant compartment is separated from the fungal compartment by double mesh system, so that only the mycelium is able to access to the different P sources. On the right side of the mesocosms is connected the EGM-4 CO$_2$ meter.

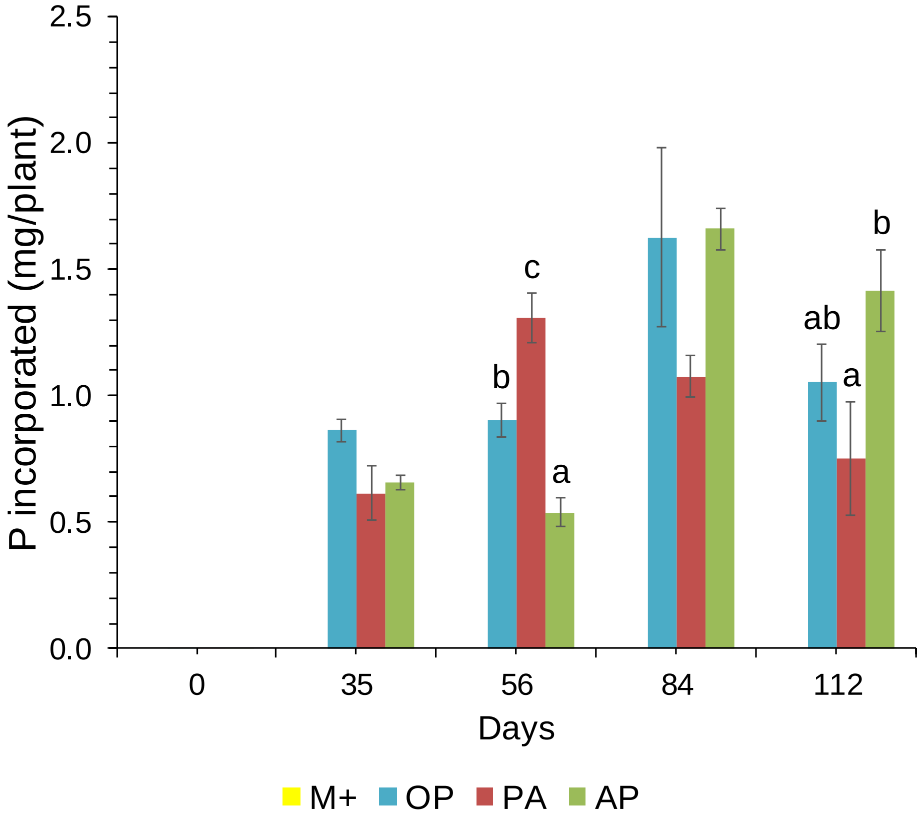


Figure 1. Phosphorus incorporated into the AM plant along the time course experiment (Mean values +/- standard error, n=3). No P was incorporated in the M+ treatment. Within each P source, treatments with significant differences are labelled with different letters ($p < 0.05$) as result of a one-way ANOVA. Abbreviations: OP, orthophosphate; AP, apatite; PA, phytic; M+, blank with no P.

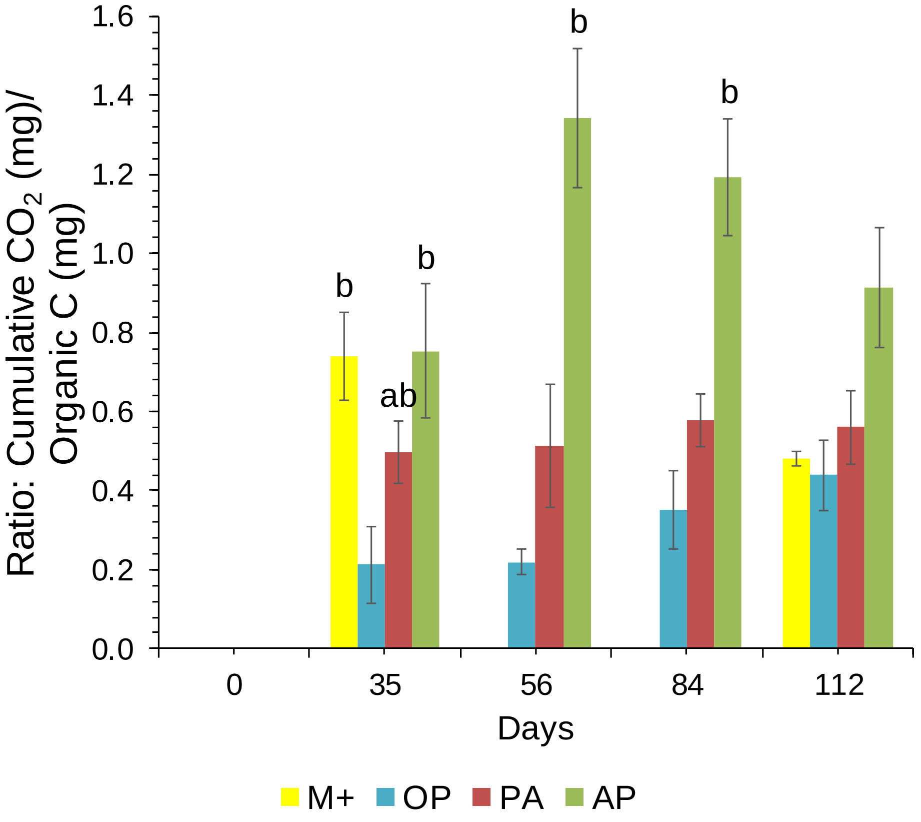


Figure 3. Ratio of respired cumulative CO_2 and the accumulated organic C for the fungal compartment containing the different P sources. Within each P source, treatments with significant differences are labelled with different letters ($p < 0.05$) as result of a one-way ANOVA. Abbreviations: OP, orthophosphate; AP, apatite; PA, phytic; M+, blank with no P.

A

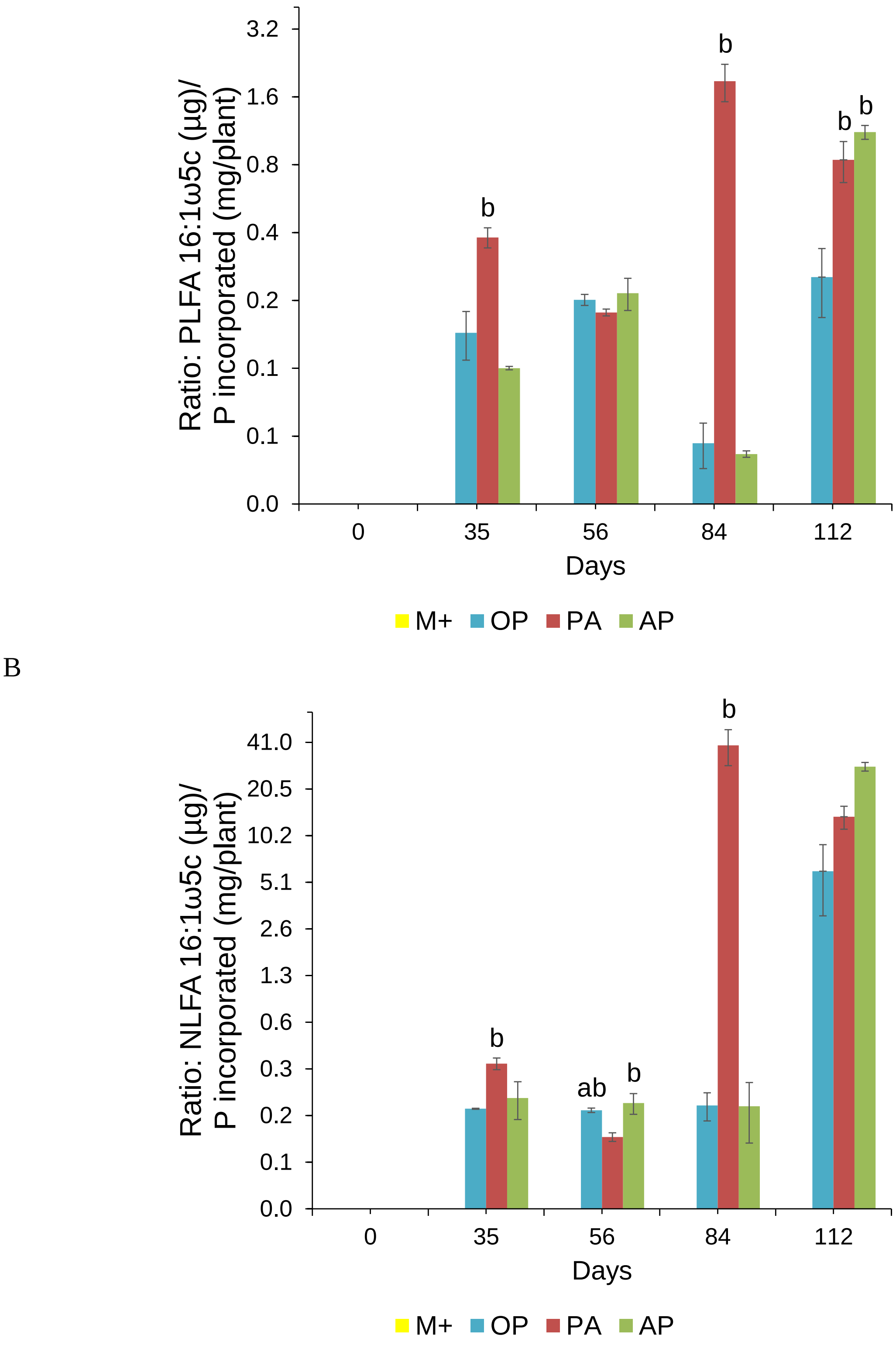


Figure 4.Mean values and standard errors (n=3) from ratios of PLFA 16:1ω5c to P incorporated within the tomato plant (A) and ratio of NLFA16:1ω5c to P incorporated within the tomato plant (B) from the different P sources along the time course experiment. Within each P source and day, treatments with significant differences are labelled with different letters (P<0.05) as result of a one-way ANOVA. Abbreviations are: OP, orthophosphate; AP, apatite; PA, phytic; M+, blank with no P.

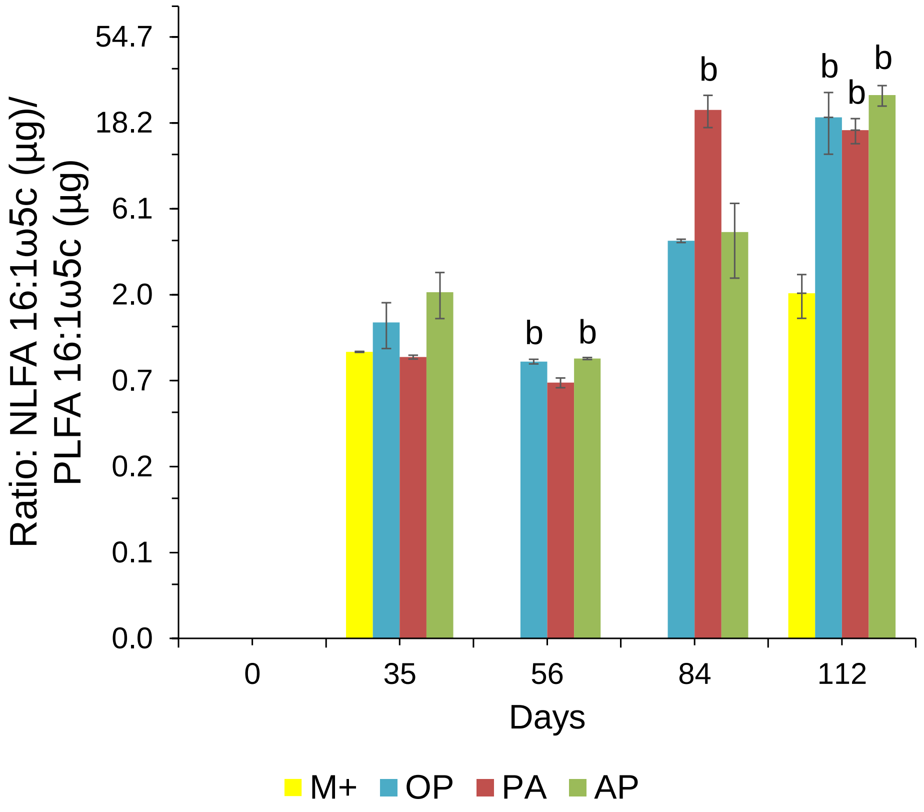


Figure 5. Ratio of NLFA 16:1ω5c to PLFA 16:1ω5c. Shown are mean values and standard errors (n=3). Within each P source, treatments with significant differences are labelled with different letters (P<0.05) as result of a one-way ANOVA. Abbreviations: OP, orthophosphate; AP, apatite; PA, phytic; M+, blank with no P.

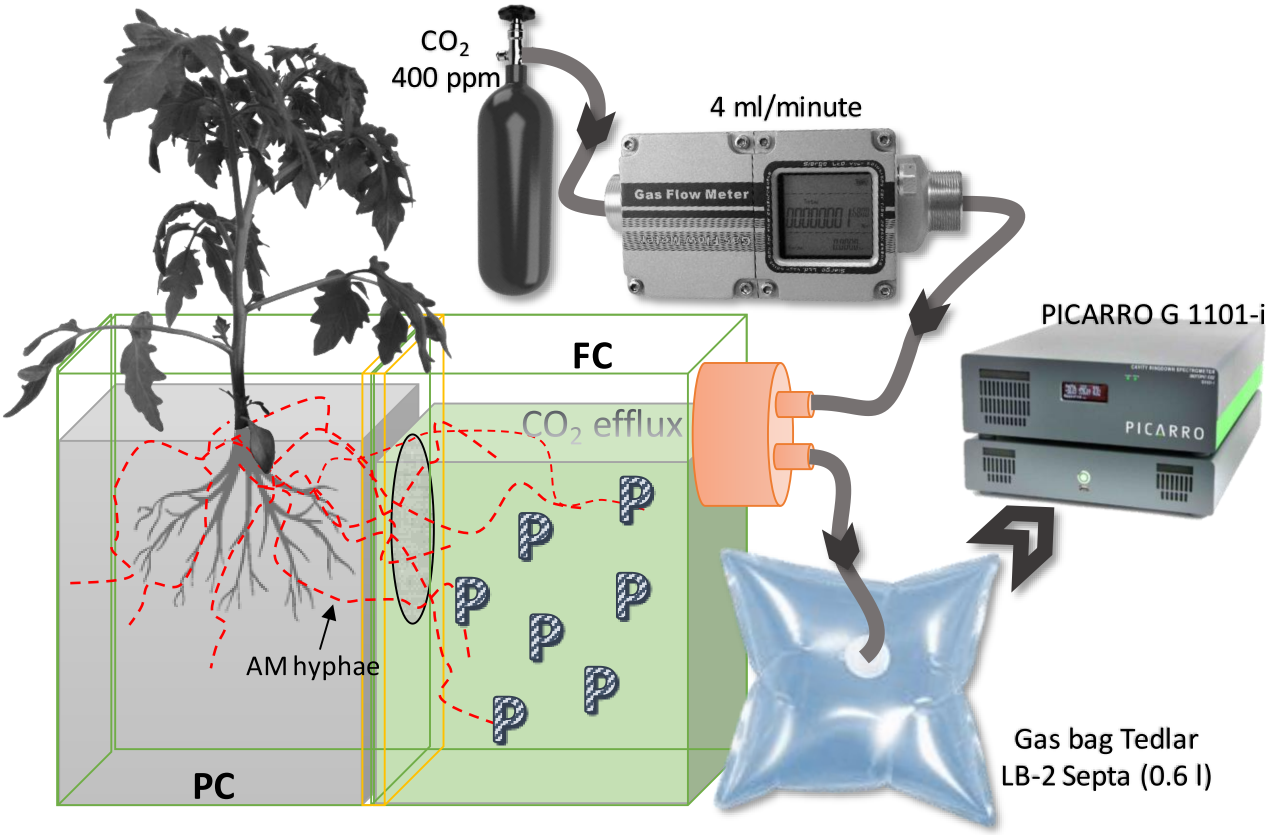


Figure 6. Diagram of the mesocosms setup used to measure the content of $^{13}CO_2$ respired at the fungal compartment and measured by the Picarro G 1101-i. The gas bags were filled with the air respired from the fungal compartment and then were measured in the Picarro G1101-i.

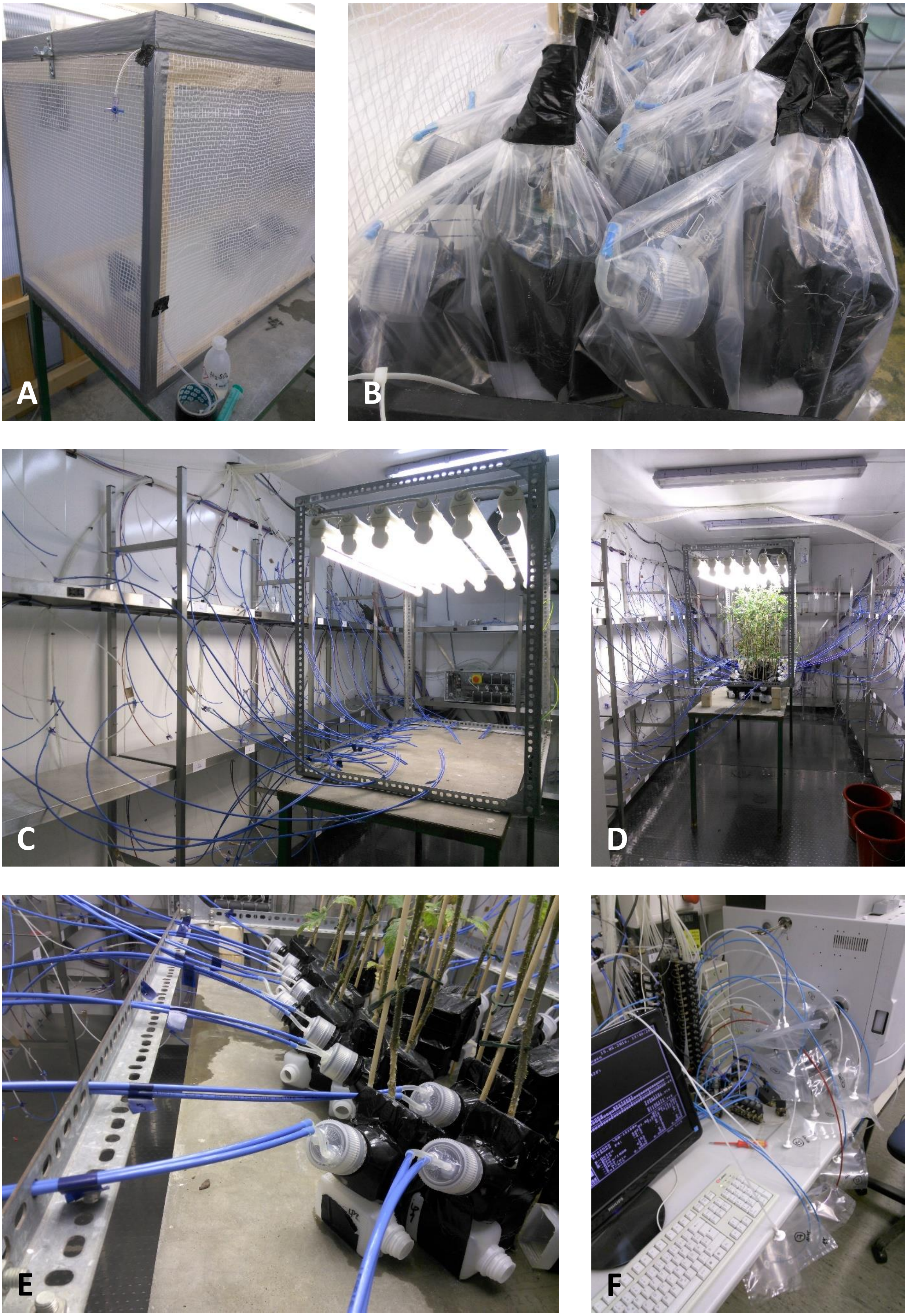

A
B
C
D
E
F

Figure 7. Image plate describing the labelling process with $^{13}CO_2$ inside the labelling box with forced ventilation to obtain a homogeneous $^{13}CO_2$ concentration inside the labelling box (A). The different mesocosms were wrapped in plastic bags (B). Inside the plant chamber tubing connections were installed (C, D, E) to carry the $^{13}CO_2$ respired by the different fungal compartments into the gas bags (F).

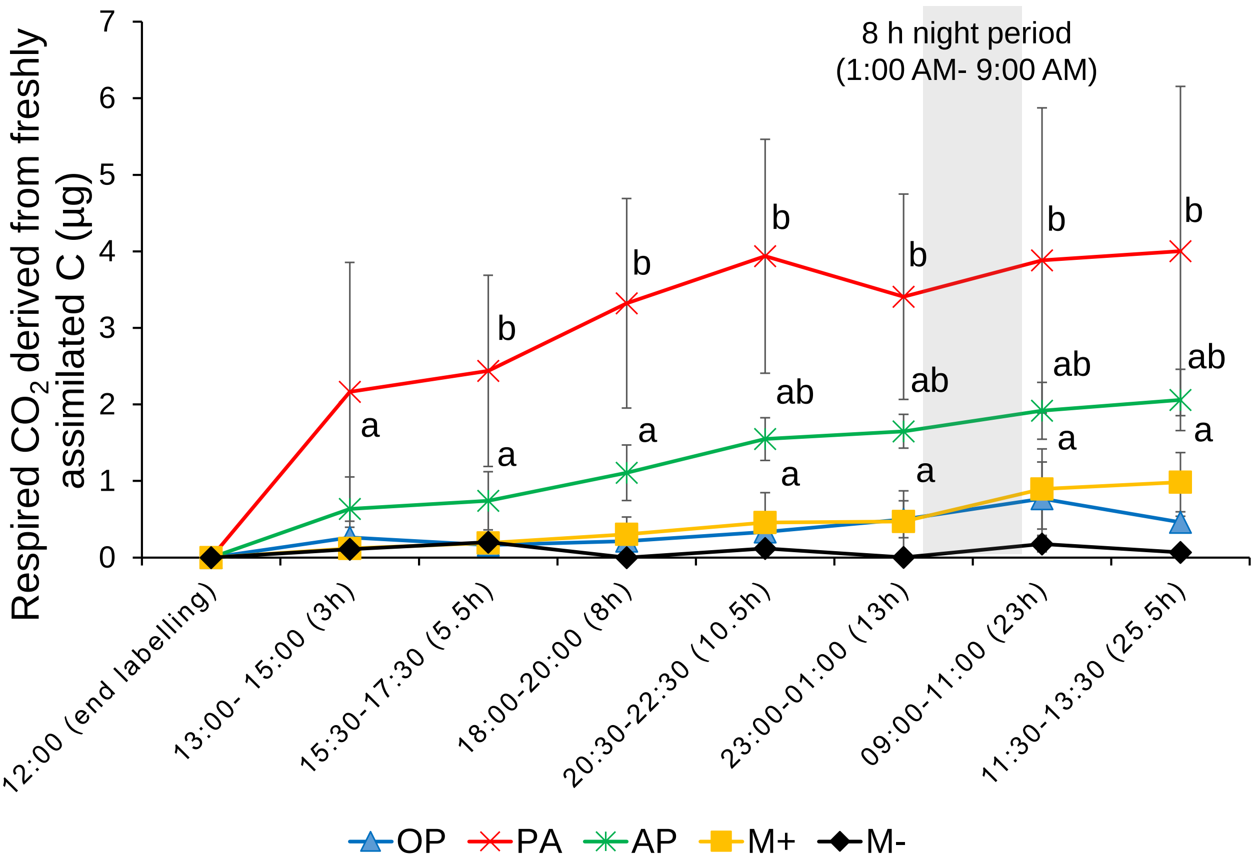
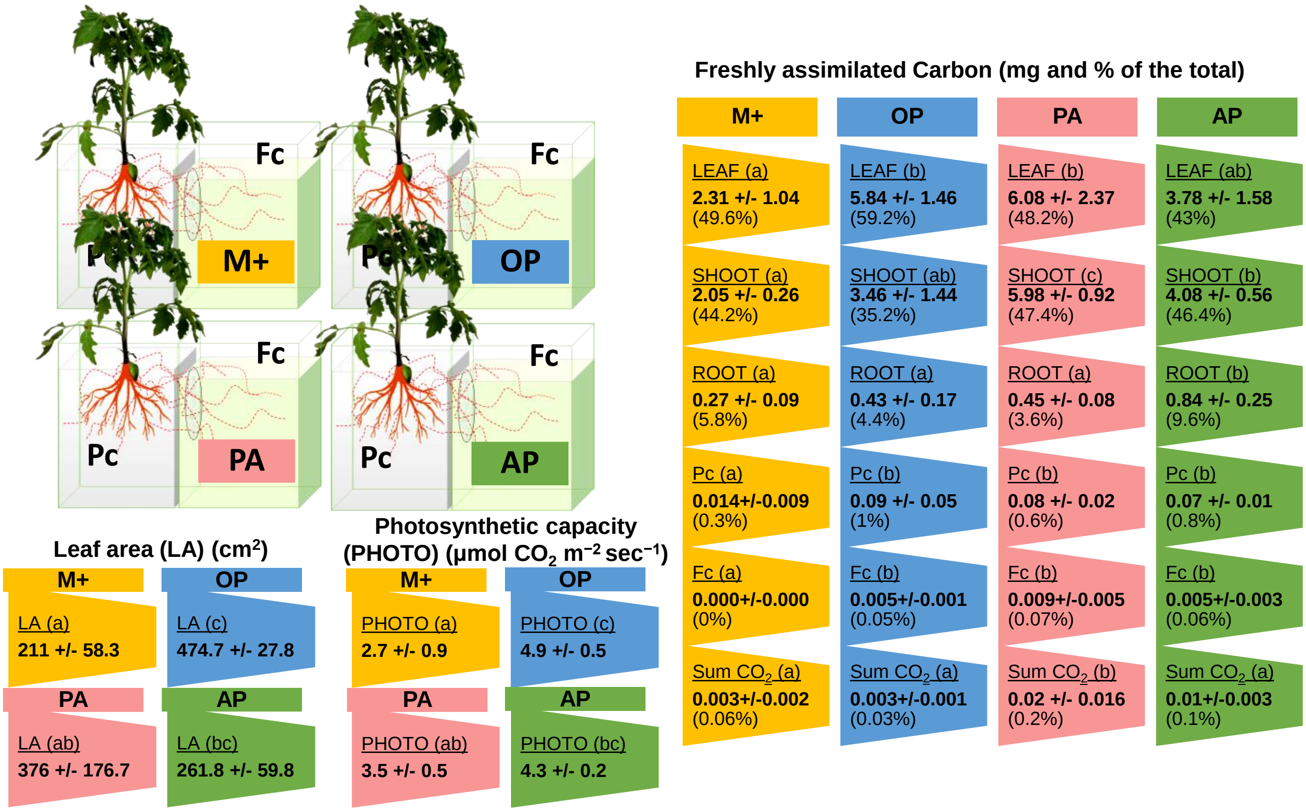


Figure 8. CO_2 respiration from the fungal compartment (µg) derived from the freshly assimilated C. The measurements were performed with the Picarro G1101-i, after a ^{13}C pulse labelling. Each line plots the mean value and standard error of the respired CO_2 (µg) of three independent mesocosms harvested at different time points for each treatment. Within each P source, treatments with significant differences are labelled with different letters ($P<0.05$) as result of a one-way ANOVA. Abbreviations: OP, orthophosphate; AP, apatite; PA, phytic; M+, blank with no P; M-, non-mycorrhizal plants.



Leaf area (LA) (cm^2)

M+	OP	PA	AP
LA (a) **211 +/- 58.3**	LA (c) **474.7 +/- 27.8**	LA (ab) **376 +/- 176.7**	LA (bc) **261.8 +/- 59.8**

Photosynthetic capacity (PHOTO) (µmol CO_2 m^{-2} sec^{-1})

M+	OP	PA	AP
PHOTO (a) **2.7 +/- 0.9**	PHOTO (c) **4.9 +/- 0.5**	PHOTO (ab) **3.5 +/- 0.5**	PHOTO (bc) **4.3 +/- 0.2**

Freshly assimilated Carbon (mg and % of the total)

M+	OP	PA	AP
LEAF (a) **2.31 +/- 1.04** (49.6%)	LEAF (b) **5.84 +/- 1.46** (59.2%)	LEAF (b) **6.08 +/- 2.37** (48.2%)	LEAF (ab) **3.78 +/- 1.58** (43%)
SHOOT (a) **2.05 +/- 0.26** (44.2%)	SHOOT (ab) **3.46 +/- 1.44** (35.2%)	SHOOT (c) **5.98 +/- 0.92** (47.4%)	SHOOT (b) **4.08 +/- 0.56** (46.4%)
ROOT (a) **0.27 +/- 0.09** (5.8%)	ROOT (a) **0.43 +/- 0.17** (4.4%)	ROOT (a) **0.45 +/- 0.08** (3.6%)	ROOT (b) **0.84 +/- 0.25** (9.6%)
Pc (a) **0.014+/-0.009** (0.3%)	Pc (b) **0.09 +/- 0.05** (1%)	Pc (b) **0.08 +/- 0.02** (0.6%)	Pc (b) **0.07 +/- 0.01** (0.8%)
Fc (a) **0.000+/-0.000** (0%)	Fc (b) **0.005+/-0.001** (0.05%)	Fc (b) **0.009+/-0.005** (0.07%)	Fc (b) **0.005+/-0.003** (0.06%)
Sum CO_2 (a) **0.003+/-0.002** (0.06%)	Sum CO_2 (a) **0.003+/-0.001** (0.03%)	Sum CO_2 (b) **0.02 +/- 0.016** (0.2%)	Sum CO_2 (a) **0.01+/-0.003** (0.1%)

Figure 9. Diagram containing the results on the parameters measured during the ^{13}C pulse labelling experiment. On the left side of the diagram the photosynthetic capacity of the plants that accessed the different phosphorus sources immediately before the ^{13}C pulse labelling experiment is shown. In bold letters inside each colored cell, the mean value and the standard error is shown (n=3) for each parameter. In the center of the diagram the leaf area for the different treatments is shown. On the right side, the colored cells inform about the freshly assimilated C at the different plant organs, the plant and fungal compartments and the cumulative CO_2 respiration from the fungal compartment (Picarro G1101-i) for the different treatments 24 hours after the ^{13}C pulse-chase labelling experiment. Between brackets the mean percentage of freshly assimilated C into the plant organs, the plant and fungal compartments and the cumulative CO_2 respiration is shown. Within each P source, treatments with significant differences are labelled with different letters between brackets ($P<0.05$) as result of a one-way ANOVA. Abbreviations: OP, orthophosphate; AP, apatite; PA, phytic; M+, blank with no P; Fc, fungal compartment; Pc, plant compartment.

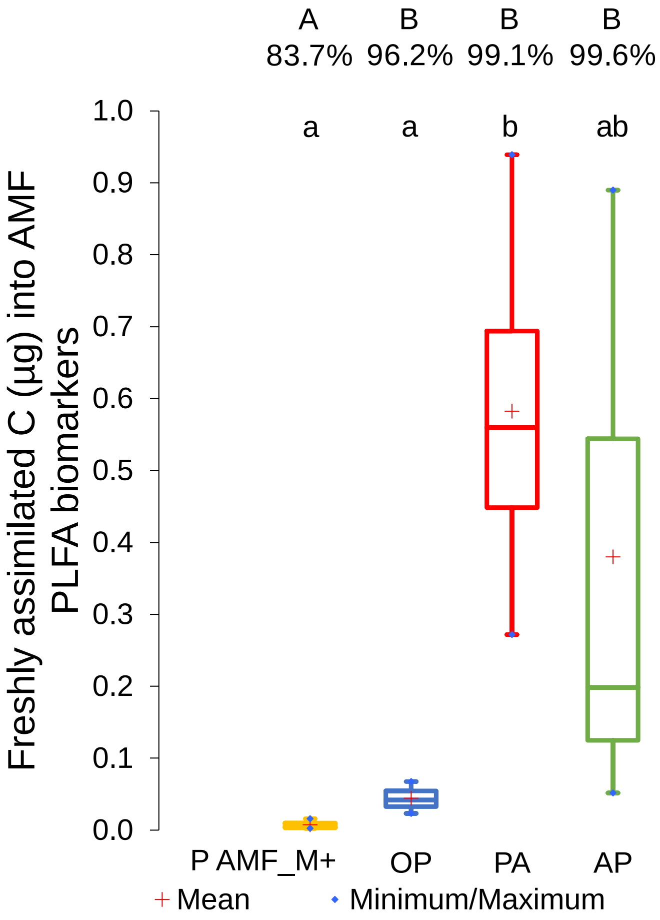


Figure 10. Box-plot showing the mean content and standard error (n=3) of freshly assimilated C (µg) into AMF PLFA biomarkers within the fungal compartment for the different phosphorus sources. Within each P source on top of each box-plot significant differences between the content of freshly assimilated C (µg) into AMF PLFA biomarkers are shown with different small letters, as result of a one-way ANOVA ($P<0.05$). Treatments with significant differences between the percentages of freshly assimilated C into AMF PLFA biomarkers are labelled with different capital letters on top of each box-plot, as result of a one-way ANOVA ($P<0.05$). Abbreviations: OP, orthophosphate; AP, apatite; PA, phytic; M+, blank with no P.

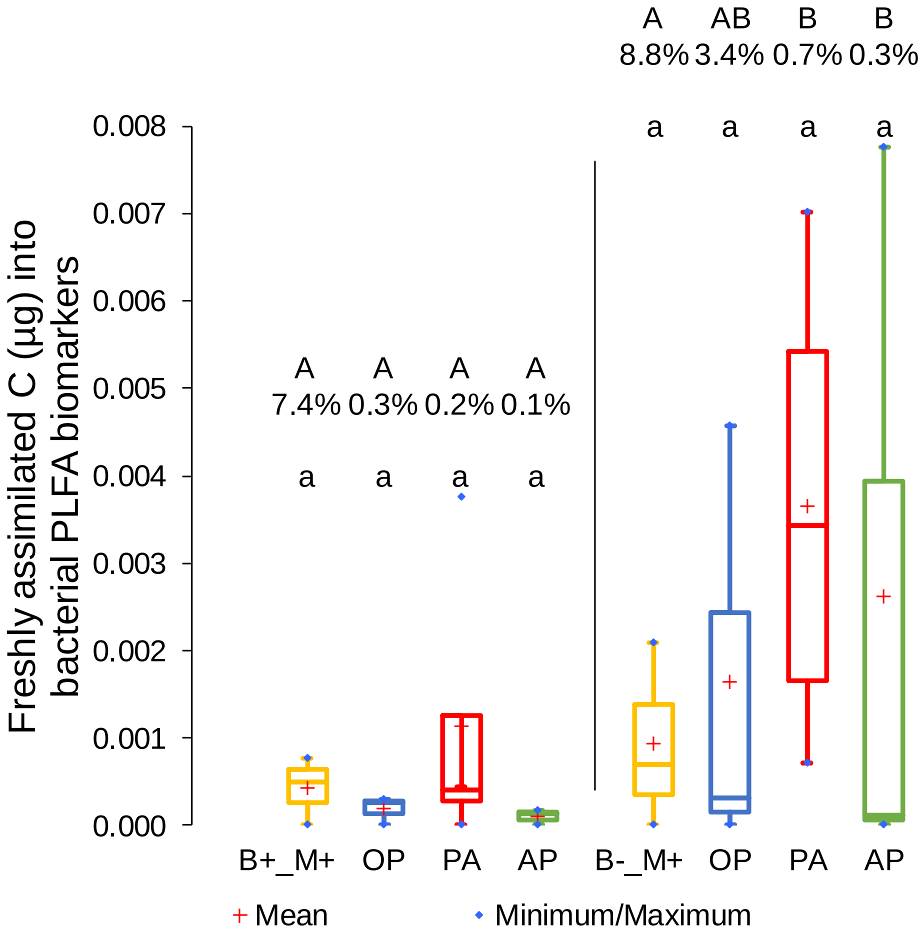


Figure 11. Box-plot showing the mean content and standard error (n=3) of freshly assimilated C (µg) into bacterial PLFA biomarkers within the fungal compartment for the different phosphorus sources. On the right side, the B+ stands for gram-positive PLFA bacterial biomarker and on the right side, B- for gram-negative bacteria. Within each P source and for each parameter on top of each box-plot significant differences between the content of freshly assimilated C (µg) for each microbial group is shown with different small letters, as result of a one-way ANOVA ($P<0.05$). Treatments with significant differences between the percentages of freshly assimilated C into each microbial group are labelled with different capital letters on top of each box-plot, as result of a one-way ANOVA ($P<0.05$). Abbreviations: OP, orthophosphate; AP, apatite; PA, phytic; M+, blank with no P.

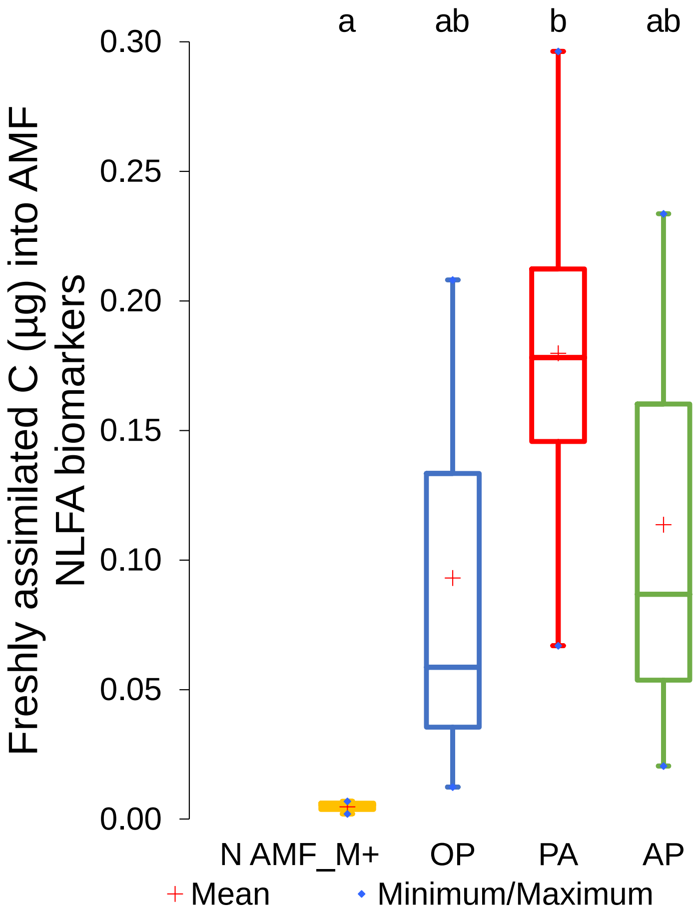


Figure 12. Box-plot showing the mean content and standard error (n=3) of freshly assimilated C (µg) into AMF NLFA biomarkers in the fungal compartments for the different phosphorus sources. Within each P source on top of each box-plot the significant differences between the content of freshly assimilated C (µg) into AMF NLFA biomarkers with different small letters is shown, as result of a one-way ANOVA (P<0.05). Abbreviations: OP, orthophosphate; AP, apatite; PA, phytic; M+, blank with no P.

3. Study II

Carbon investment required for the mobilisation of inorganic and organic phosphorus bound to goethite by an arbuscular mycorrhiza (*Solanum lycopersicum x Rhizophagus irregularis*)

Alberto Andrino[1], Jens Boy[1], Robert Mikutta[2], Leopold Sauheitl[1], and Georg Guggenberger[1]

[1] Institute of Soil Science, Leibniz Universität Hannover, Herrenhäuser Straße 2, 30419 Hannover, Germany

[2] Soil Science and Soil Protection, Martin Luther University Halle-Wittenberg, von-Seckendorff-Platz 3, 06210 Halle (Saale), Germany

Author contribution

JB, GG, AA, and RM designed the experiment. AA prepared the plant and fungal material. AA conducted the experiment and analysed the data. JB, LS, GG, and RM supervised the research. AA wrote the paper with contributions from JB, GG, LS and RM.

Submitted: *Frontiers in Environmental Science*, special research topic *Rhizosphere Functioning and Structural Development as Complex Interplay between Plants, Microorganisms and Soil Minerals.*

Abstract

Nutrient supply in phosphorus (P)-limited ecosystems, with most P being associated with secondary minerals, has to rely on efficient nutrient allocation strategies, such as those involving mycorrhizal symbioses. Yet, little is known about the extent of photo-assimilate transfer to the fungal partner, who in turn mobilises mineral-bound P sources required by the plant. This study aims to explore the carbon (C)–P trade between an arbuscular mycorrhizal (AM) plant and its ability to incorporate P from differently accessible P sources. We compared P uptake rates of AM plants for orthophosphate (OP) and phytic acid (PA), applied to mesocosms in either dissolved form or bound to goethite (α-FeOOH). The design of the mesocosms allowed the plant to only access the P in the fungal compartment via the AM hyphae. We hypothesized the AM plant to invest more C into the symbiosis, if P is present in the less accessible form. To estimate the C budget of the symbiosis, we determined total organic carbon (OC), 16:1ω5c phospholipid fatty acid (PLFA; AM fungi extraradical mycelium), 16:1ω5c neutral lipid fatty acid (NLFA; AM fungi energy storage), and CO_2 cumulative respiration in the fungal compartment. A ratio to the total C mobilised into the fungal compartment (OC+CO_2 cumulative respiration) and the P incorporated into the AM plant (Total C/P) was calculated to estimate the C investment made by the AM plant into its symbiotic partner. AM plants incorporated P derived from all four P sources exclusively via the mycorrhizal pathway in different amounts and kinetics. The Total C/P ratio was significantly larger for those AM plants accessing the goethite-bound P compounds. They also transferred significantly larger amounts of PLFA and NLFA to their fungal partner, both indicating a larger plant C investment per P taken up. Our data provide first evidence about the ability of an AM plant to incorporate P from an organic source bound to a secondary mineral. The different C investments of AM plants into P allocation from variably available sources suggests a broad nexus between P mining strategies, resource partitioning in soil, and the amounts of C accumulated in terrestrial soils.

Keywords:

Soil, resource partitioning, Goethite, organic phosphorus, inorganic phosphorus, arbuscular mycorrhiza, carbon-phosphorus trading, PLFA 16:1ω5c, NLFA 16:1ω5c.

Introduction

Phosphorus (P) is an essential element for plant growth and productivity. While excessive utilization of P as fertilizer has led to widespread eutrophication of inland and coastal waters

(Rowe et al., 2016), its deficiency is still a major constraint to agricultural productivity, affecting an estimated area of >20 million km^2 worldwide (Oberson et al., 2001). This is especially true for tropical soils where secondary minerals immobilise P to a large extent. In 2014, phosphate rocks were included in the list of the twenty critical raw materials of the European Commission (George et al., 2016), suggesting that this finite resource could be exhausted within this century (Gilbert, 2009). Consequently, all plant strategies which increase P uptake and improve its use-efficiency will be increasingly valuable to prevent P loss to adjacent ecosystems and lower the need of P fertilizers (Smith and Smith, 2011).

Plants can only take up P as free phosphate anions, either as $H_2PO_4^-$ or as HPO_4^{2-} (Becquer et al., 2014). Phosphate concentrations in soil solutions span from 1 to 10 µM, being about 1000-fold smaller than cellular contents in plants, thus often insufficient for optimal growth (Smith and Smith, 2012). Soil P occurs in forms of varying accessibility to plants with >90% of it being bound in plant-inaccessible forms (Mengel and Kirkby, 2001). Phosphorus occurs either as inorganic phosphates, primarily in associations with Ca, Fe, and Al, or in organic forms. Organic P constitutes 20–80% of total soil P and includes phosphomonoesters such as inositol phosphates (IP), phosphodiesters as ribonucleic acid, deoxyribonucleic acid, lipoteichoic acid, phospholipid fatty acids, and organic polyphosphates, e.g. adenosine triphosphates. The most prevalent phosphomonoester is myo-IP6, which contains six phosphate groups bound to cyclohexane and has nine stereoisomers. The myo-IP6 stereoisomer or phytic acid (PA) is the most common form of inositol phosphates and represents >50% of the organic P in soils (Nash et al., 2014; Ognalaga et al., 1994).

Phosphorus deficiency of plants is caused by strong adsorption of inorganic and organic P to Al and Fe hydroxides and oxides (summarized as "oxides"), making large proportions of total P unavailable to plants (Javaid, 2009; Osorio and Habte, 2001). The specific adsorption and high affinity to soil oxides is greater for the organic P forms (He and Zhu, 1998). As compared to inorganic P forms, the dynamics of organic P species in soils are much less investigated. This is surprising since this fraction is highly relevant to the supply of P to crops in deeply weathered soils like Oxisols (Rodrigues et al., 2016). To overcome soil P limitation, plants developed different strategies to acquire P from soil solution. One strategy is to increase the exchange-surface of the root–soil interface; another strategy lies in solubilizing complexed P by root exudates, such as low-molecular-weight organic acids and phosphatases. The most widespread strategy nevertheless is to rely on symbiotic associations with mycorrhizal fungi and P-solubilizing or organic P-mineralizing bacteria (Giles et al., 2012; Richardson et al., 2009).

In P-deficient environments, e.g. Spodosols, Ultisols, or Oxisols (Yang et al., 2013), selection pressure commonly results in the proliferation of free-living microorganisms and symbiotic associations with mycorrhizal fungi which have the potential to mobilise and mineralize unavailable organic P (Nash et al., 2014). Most terrestrial plants form symbiosis with one or more kinds of mycorrhizal fungi. About 80% of plant species are associated with arbuscular mycorrhizal fungi (AMF) (Cairney, 2000; Johnson, 2010; Smith et al., 2008), which enhance the plant's access to limiting belowground resources (Read, 1991).

Plants that establish symbiosis with an AMF can acquire part or almost all of the phosphorus for their metabolic activities (Javot et al., 2007; Lambers et al., 2015; Willis et al., 2013). As they are able to mine soils for P, research on P release rates from secondary minerals is important to improve models on plant nutrient cycling by AMF (Cardoso et al., 2006). Though some studies have shown that mycorrhizal and non-mycorrhizal plants seem to use the same labile P sources (Bolan et al., 1984; Frossard et al., 2011; García, 2000), others demonstrated that mycorrhizal plants obtain P from usually unavailable sources of organic and inorganic P (Rychter et al., 2016). In addition, Bolan et al. (1987) proposed that AMF may cleave the bond between Fe and P and thus release P without depicting the underlying mechanisms .

Turner (2008) suggested that the different soil P species constitute a gradient of biological availability based on the plant's investment to access the phosphate. For example, phytate, being most resistant to hydrolysis, is hypothesized as the metabolically most expensive P source. This makes necessary to understand if there exists a C-P trading in arbuscular mycorrhizal symbiosis.

In return for providing almost all P needed by the AM plant, the fungus receives up to 20% of net plant photosynthates under ambient atmospheric CO_2 (Pfeffer et al., 2004). The C assimilates are transported mainly as lipid droplets and glycogen to the AM fungal hyphae (Bago et al., 2002). At least three publications have recently shown that AMF do not have the capacity to produce fatty acids completely on their own (Bravo et al., 2017; Keymer et al., 2017; Luginbuehl et al., 2017). Instead, AMF were found completely depending on plant-derived fatty acids. These studies suggest a model in which a chain length of 16 carbons (C16:0) b-monoacylglycerol molecules are transported from the root cell through the periarbuscular membrane to the fungus. Fatty acids of chain lengths up to C16 are needed, because the fungus apparently lacks genes encoding multi-domain cytosolic fatty acid synthase subunits. This makes fatty acids a major good of trade between plant and fungus in AM.

To compare the true costs of incorporating different P forms into the plant by the AMF pathway requires the quantification of the C fluxes. The development of AM itself already presents a complex series of trade-offs between the C cost of the fungus and the benefits of enhanced nutrient supply to the plant (Cavagnaro et al., 2008). Quantification of the fluxes in mycorrhiza is one of the most important, yet little explored tasks of mycorrhizal physiology and ecology. Monitoring the respired CO_2 as the end product of the mycorrhizal catabolism is one essential part for the calculation of the full C budget of the mycorrhizal symbiosis (Slavikova et al., 2016). In order to identify the behaviour of AM in mobilising P from differently accessible P forms, we carried out a mesocosm experiment under controlled conditions. Our work aimed at (i) clarifying whether the AM plant can take up P from less accessible sources and (ii) to determine whether a trading mechanism between C and P over the AM exists. We hypothesize that less accessible P sources cause larger photoassimilate investments by the plant partner, resulting in differing kinetics of the AMF providing P to the host plant.

Materials and methods

Plant Growth and Mycorrhization

We selected the AM mycorrhizal association between *Solanum lycopersicum* L. (var. Moneymaker) x *Rhizophagus irregularis* (DAOM 197198) for our experiment. The association has been previously investigated to elucidate processes related with the nutritional benefits offered by the AMF to the tomato plant (Giovannetti et al., 2012; Nagy et al., 2005; Schaarschmidt et al., 2006). The reason for using the AMF *R. irregularis* is due to its global distribution and adaptation to intensive agricultural practices. This ubiquitous occurrence indicates that *R. irregularis* is compatible with a wide range of soil conditions like pH (5.6–8.0), P availability (0.3–18.8 mg/kg), sand content (17.5–57.0%), and C content (1.0–10.5%) (Köhl et al., 2016). *R. irregularis* DAOM 197198 (syn: *Glomus irregulare*) recently reassigned from *Glomus intraradices* Schenck and Smith (Krüger et al., 2012) was used as the mycorrhizal inoculum. It consisted of 0.4 g of spores, hyphae, and root fragments from a *Sorghum bicolor* trap plant culture (Brundrett, 1996). Seeds of *Solanum lycopersicum* L. var. Moneymaker (Volmary GmbH) were surface sterilized with 5% H_2O_2 for 10 minutes and washed three times with sterile distilled water. Seeds were germinated on petri dishes for 3 days at 27°C.

Moneymaker germinated seeds were sowed on 75 ml pots QP96 (HerkuPlast Kubern GmbH) containing the AM inoculum and 70 ml autoclaved acid washed quartz sand, which has been successfully used as plant and fungal growth substrate before (Johansen et al., 1996; Olsson and Johansen, 2000). Allowing the AM mycelium to grow from a colonized root into purified quartz sand, a relatively pure mycelium could be extracted and used for our studies. Mycorrhized and non-mycorrhized control tomato plants were grown in a greenhouse (16/8 light/dark, 24/20°C light/dark, 50–60% relative humidity, photon flux density of 175–230 µmol/m/s). Seedlings were watered every day with 10 ml deionized water and were fertilized with 5 ml low P (0.32 mM) Long Ashton nutrient solution pH 6.5 (Hewitt, 1966) on alternate days. The complete root system of 10 individuals was processed to test for the presence of arbuscular mycorrhiza in the roots of four week old plants before transplanting. A root subsample was digested with 10% KOH (35 min, 95°C) and stained using the ink and vinegar staining technique for AMF (Vierheilig et al., 1998b). Stained root fragments were mounted on glass slides and observed at 400× magnification using an Olympus BH2 microscope (Olympus Optical Company Ltd, Tokyo, Japan), to determine the mycorrhizal status using the methodology from McGonigle et al. (1990), before the plants were transplanted to the mesocosms.

Mesocosm Experiment

Each mesocosms consisted of a plant compartment connected to a fungal compartment in which only the mycelium was able to develop and access the different P sources due to two barriers (Figure 1). Each mesocosms was fabricated from a Nalgene® 250 ml square polypropylene bottle cut at 45° angle. The threaded section of the bottle was glued with Pattex ® hot glue on the side with the largest surface of the bottle base. A second Nalgene®100 ml square polypropylene bottle was cut into two sections, one as irrigation compartment (30 ml) and the second as support for the mesocosms. Through a 3 mm diameter perforation at the base of the plant compartment, a 3 mm diameter fiberglass wick (Ortmann Kapillarbewässerung, Vlotho, Germany) was passed through, leaving 5 cm of the wick inside the irrigation compartment, where twice a week 10 ml deionized water were applied (Supplementary Figure 1). The wick irrigation system operates in a closed cycle, without runoff and covers maximum water requirements of the plant (Kuntz, 2013; Son et al., 2006). At the plant compartment side a polyamide mesh with a pore size of 20 µm and 30 mm side (Franz Eckert GmbH) separated mycorrhizal roots and mycelium (Fitter et al., 1998; Watkins et al., 1996). The second barrier was a 25 mm diameter polytetrafluoroethylene membrane with a pore size of 5–10 µm (Pieper

Filter GmbH). The second membrane allowed the AM hyphae to cross but prevented mass flow and diffusion of ions into the plant compartment (Mäder et al., 1993, 2000; Vierheilig et al., 1998a). Hence, P sources were exclusively accessible to the plant by the hyphae. There were no other P sources present in the system as the cultivation substrate in the plant compartment consisted of acid washed quartz sand (1–2 mm) free of nutrients (data not shown). Four-week-old mycorrhized and non-mycorrhized seedlings were transplanted into each mesoscosm and placed in a glasshouse under controlled climatic conditions (24/20°C light/dark; photoperiod 16/8 h light/dark; 50–60% relative humidity; photon flux density of 175–230 µmol/m^2/s).

Once a week all mesocosms were rotated to achieve homogeneous growth conditions and were fertilized twice a week on top of the plant substrate with 5 ml no P Long Ashton nutrient solution at pH 6.5 (Hewitt, 1966). The P sources offered in the fungal compartment, including the control treatments, are summarized in Table 1. The 55 ml volume of the fungal compartment was amended by a total P amount of 30 mg and water volume was adjusted to field capacity. The water volume at the fungal compartment was determined with a time-domain reflectometry probe Trime Pico connected to a Trime-FM version P2 (Imko Micromodultechnik GmbH, Ettlingen, Germany). The treatments containing quartz sand as a substrate (60 g) in the fungal compartment exhibited 19% water volume while the ones containing goethite (24.3 g) exhibited 41% water volume. The water volume was checked once a week and amended with autoclaved MilliQ water to field capacity. A nylon black cloth with a 1 mm pore was placed on the mouth of the fungal compartment to prevent algae growth, water evaporation and to facilitate gas diffusion to the outside of the fungal compartment (Supplementary Figure 1).

The P uptake and C investment was tracked for 91 days. The first harvest point was on the day of transplanting (day 0) to determine the P stocks of the plants (n=5), followed by another six sampling points at days 7, 21, 35, 49, 77, and 91. In each of the six harvest points, three biological replicates of each treatment were processed.

Preparation of Phosphorus Sources and Phosphorus Desorption

Each P source was placed inside each fungal compartment as explained in Table 1. The orthophosphate (OP) source was added as KH_2PO_4 (Sigma Aldrich, Taufkirchen, Germany) and the organic P source was added as phytic acid (PA) sodium salt hydrate (Sigma Aldrich). Two adsorption complexes were produced using the OP and the PA bound to goethite. The adsorption complexes were prepared equilibrating the P compounds with goethite (Bayferrox 920 Z). First, 50 g of goethite were equilibrated for 16 h in 250 ml MilliQ H_2O adjusted to pH

4. Then, 250 ml MilliQ H_2O containing either 17 g KH_2PO_4 or 0.7191 g $C_6H_{18}O_{24}P_6$ were added to the goethite suspension and equilibrated for 48 h on an overhead shaker. The goethite-P suspensions were centrifuged (3000 × *g*, 15 min) and the pellets were subsequently washed with MilliQ H_2O until the electric conductivity was <40 µS/cm. The resulting goethite-P associations were shock-frozen in liquid N_2, and freeze-dried. The OP and PA bound to the goethite adsorption complexes was determined in triplicate by hydrolysing 5 mg of the goethite-P associations in concentrated HNO_3 and subsequent measurement of P contents by ICP-MS Agilent 7500C (Agilent Technologies Ireland Ltd., Cork, Ireland). The adsorption complexes contained 1.24 mg P/g in case of GOE-OP and 1.79 mg P/g for GOE-PA, respectively. A second analysis on the adsorption complexes was carried out to determine the amount of desorbable P, by shaking 1.0 g of each goethite-P association (n=3) in 30 ml of MilliQ water for 24 h on an overhead shaker. The centrifuged supernatant (3000 × *g*, 15 min) was filtered through a 0.45 µm syringe filter (PVDF) and 1 ml was mixed with 1 ml 30% HNO_3 and the mixture was filled up to 10 ml with MilliQ water and P content was then measured by ICP-MS.

Plant and Fungal Phosphorus Contents

At each of the six harvest point, shoots and washed excised roots were air dried (70°C, 48 h), weighed and ball milled. Plant samples were incinerated at 480ºC for 8 h, digested with 1 ml 30% HNO_3, filtered and analysed by ICP-MS. The P stocks were determined for the whole plant and the amount of total P incorporated by the AM plant over time was calculated as the difference of the P stock at each harvest point and the P stock initially present in the transplanted plant on day 0 (determined from five subsamples). The separation of hyphae/spores took place on day 91 following a modified method of Brundrett (1996). First, 4 g of goethite-P associations (GOE-OP, GOE-PA) or 10 g of sand (OP, PA) were sampled from each fungal compartment in 50 ml Falcon test tubes. Tubes were then filled with a fixative solution (0.9 g/l NaCl plus 3% glutaraldehyde), equilibrated for 2 h on an overhead shaker, and then centrifuged (3000 × *g*, 15 min). The supernatant was filtered through a polyethersulfone 0.45 µm filter (Supor® PES membrane disc filters, Pall Life Sciences, Hampshire, UK), using a vacuum pump system. Pellets were re-suspended in a 50% sucrose solution by vigorously shaking plus an equilibration step (1 hour) on an overhead shaker. The samples were then centrifuged for 15 minutes at 3000 × *g* to facilitate the separation of hyphae and spores in the glucose density gradient. Immediately after centrifugation, hyphae and spores in the sucrose supernatant were poured onto the same polyethersulfone 0.45 µm filter and carefully washed with MilliQ H_2O to remove the sucrose and the fixative solution. This step was repeated three times per sample. After rinsing the hyphae and spores, the filters were placed in petri dishes. The hyphae/spores were collected under a stereomicroscope with a glass pipette and deposited in 2 ml Eppendorf tubes. The fungal material was air-dried at 50°C for 96 h. The P content (P mg /hyphae mg) was determined for an aliquot of fungal material. Samples were incinerated at 480ºC for 8 h, digested with 1 ml 30% HNO_3, filtered, and analysed by ICP-MS.

Organic Carbon and Carbon Dioxide Production at the Fungal Compartment

At each harvest point, 2 g from each fungal compartment were air-dried (70°C, 48 h), weighed, and ball milled. The C content of these samples was measured using an Elementar vario MICRO cube C/N analyser (Elementar GmbH, Hanau, Germany). For the calculation of the C stock in the fungal compartment it was multiplied the C content (mg/g) determined at the analyser by the total weight of substrate in the fungal compartment reported in Table 1. As there was no inorganic C in the fungal compartment, all C was considered as organic carbon (OC).

The CO_2 efflux (mmol CO_2 m^2 h^{-1}) was measured in the fungal compartment twice a week using an EGM-4 infrared gas analyser (PP-systems, Hitchin, UK) as a close dynamic system (Vermue et al., 2008). At each measuring point, the black cloth was removed, the airtight lid was placed in the mouth of the fungal compartment and the inlet and outlet tubes were connected to the EGM-4 (Supplementary Figure 1). CO_2 efflux was automatically calculated for a headspace volume of 40 ml and an exposed area of 0.0012 m^2 of the fungal compartment. The CO_2 content was measured every 4.8 s and the CO_2 flux was measured for at least 3 min or until a good quadratic fit was obtained. Cumulative CO_2 production was calculated in mg by interpolation using a cubic spline function (Gentsch et al., 2018) for each fungal compartment.

At each time point, the Total C/P ratio was used to evaluate and estimate the investment made by the AM plant into its symbiotic partner at the fungal compartment per plant P incorporated (Eq.1), since all the C measured at the fungal compartment, in the form of total OC and cumulative CO_2 will be exclusively carried by the AMF.

$$\text{Total C/P} = \frac{(\text{OC (mg)} + \text{cumulative CO}_2\text{ (mg)})}{\text{P incorporated (total mg per plant)}} \tag{1}$$

Where OC is the total C (mg) per fungal compartment; cumulative CO_2 is the total cumulative respired CO_2 (mg) at the fungal compartment.

Fatty Acid Analysis and *R. irregularis* Biomass Estimation

Using a chloroform-methanol-citrate buffer (1:2:0.8 v/v/v), lipids were extracted twice from 16 g of fungal compartments containing quartz sand or 8 g for the ones with goethite. Thereafter, extracts were fractionated by solid phase extraction with activated Silica gel (Sigma Aldrich, pore size 60 Å, 70–230 mesh) into neutral lipid fatty acids (NLFA), glycolipids, and phospholipid fatty acids (PLFA) by elution with 5 ml of chloroform, 20 ml of acetone, and 20 ml of methanol, respectively. The PLFA and NLFA samples were subjected to mild alkaline methanolysis, which transformed the neutral lipids and the phospholipids into free fatty acid methyl esters, as outlined in Frostegård et al. (1991) with modifications by Bischoff et al. (2016). The methyl esters were then separated by gas chromatography using an Agilent 7890A GC system (Agilent Technologies Ireland Ltd., Cork, Ireland) equipped with a 60 m Zebron capillary GC column (0.25 mm diameter and 0.25 µm film thickness; Phenomenex, Torrance, California, USA) and quantified with a flame ionization detector, using He as carrier gas. Glyceryl tridodecanoate (25 µg) and nonadecanoic acid (25 µg) were used as internal standards during the extraction and tridecanoic acid methyl ester (15 µg) was added to each sample and standard before analysis as a recovery standard. Identification of the fatty acids was achieved by use of the relative retention times, in comparison to that of the internal standard using the GC ChemStation (B.03.02.341) software (Agilent Technologies Ireland Ltd., Cork, Ireland).

The AMF *R. irregularis* DAOM 197198 model organism (Daubois et al., 2016) has a fatty acid composition ranging from C16:0 to C22:2 with 16:1ω5 as major fatty acid (Aarle and Olsson, 2003; Calonne et al., 2010; Wewer et al., 2014). The PLFA 16:1ω5c can be used for evaluating the amount of extraradical mycelia of AMF, making it a good predictor for the amount of C allocated to AMF (Aarle and Olsson, 2003; Marschner, 2007; Olsson and Johansen, 2000). In addition, the NLFA 16:1ω5c is considered a good predictor on energy storage by the fungus (Bååth, 2003). NLFA are stored in intraradical vesicles, spores, extraradical mycelium and make up a large proportion of the AM fungal biomass. They are metabolized in the mycelium through the glyoxalate cycle, and might provide the major fungal energy source as respiratory substrate (Aarle and Olsson, 2003; Olsson and Johnson, 2005; Olsson and Wilhelmsson, 2000). Therefore, PLFA and NLFA 16:1ω5c were analysed to assess the biomass and energy storage of AMF in the fungal compartment, respectively (Johansen et al., 1996; Larsen et al., 1998; Olsson et al., 1997, 2002; Stumpe et al., 2005). Ratios of 16:1ω5c PLFA to plant P uptake (Eq. 3) and NLFA to plant P uptake (Eq. 4), were used to estimate the investment made by the tomato plants into their fungal partner in either biomass or energy storage to obtain P from each source respectively. The NLFA 16:1ω5c/PLFA 16:1ω5c ratio

(Eq. 5) was calculated as an index for the growth strategy of *R. irregularis* (Green et al., 1999; Rinnan and Bååth, 2009). For each P source, the mean NLFA 16:1ω5c/PLFA 16:1ω5c ratio was calculated for sampling points belonging to the periods where no P incorporation was detected in the AM plant tissue as well as for those where we detected P in the AM plant.

$$\text{PLFA 16:1}\omega\text{5c/P} = \frac{\text{PLFA 16:1}\omega\text{5c (total } \mu\text{g at the fungal compartment)}}{\text{P incorporated (total mg per plant)}} \tag{3}$$

$$\text{NLFA 16:1}\omega\text{5c/P} = \frac{\text{NLFA 16:1}\omega\text{5c (total } \mu\text{g at the fungal compartment)}}{\text{P incorporated (total mg per plant)}} \tag{4}$$

$$\text{NLFA/PLFA} = \frac{\text{NLFA 16:1}\omega\text{5c (total } \mu\text{g at the fungal compartment)}}{\text{PLFA 16:1}\omega\text{5c (total } \mu\text{g at the fungal compartment)}} \tag{5}$$

A high NLFA 16:1ω5c/PLFA 16:1ω5c ratio indicates preferential C allocation to storage products in form of neutral lipids. Moreover, the ratio identifies the origin of the 16:1ω5c fatty acid. At NLFA 16:1ω5c/PLFA 16:1ω5c>1, the majority of this fatty acid is considered to originate from AM fungi and not from bacteria (Cozzolino et al., 2016; Hammer et al., 2011a; Olsson, 1999; Vestberg et al., 2012). To estimate the amount of AMF grown in the fungal compartment, we used a conversion factor of 1.2 nmol PLFA 16:1ω5c per mg dry hyphae (Olsson and Johansen, 2000; Olsson and Wilhelmsson, 2000; van Diepen et al., 2010). The estimated total AM biomass was used together with the fungal P content to infer the P stock incorporated by the AMF.

Data Analysis

The normality of the data was verified with the Shapiro-Wilk’s test and homogeneity of variances using the Levene's test. One-way ANOVA analysis of the variance and the Tukey post-hoc test were employed to test for differences of mean values ($P<0.05$) of measured variables between the treatment groups, presented by the different P sources offered in the fungal compartment. Data analysis was performed using SPSS v.24 for Windows (IBM Corporation, 2016).

Results

P derived from each offered source was incorporated by the AM tomato plants, while none of the controls (GOE, M+ and M-; Table 1) showed P incorporation (Figure 2). Those AM plants which had access to OP incorporated the highest amount of P, followed by PA, GOE-OP, and GOE-PA. The first P incorporation was detected at day 49 in case of the treatments offering OP and PA and at day 77 in case of GOE-OP and GOE-PA as P sources. At day 91, AM plants reached the maximum P incorporation, with P taken up in the following sequence: OP (30.4% of the initially added P) > PA (10.4%) > GOE-OP (5.9%) > GOE-PA (2.1%) (Figure 3).

Mycorrhizal plants with access to OP had significantly larger P contents in their hyphae and spores, followed by plants supplied with GOE-OP and PA. The GOE-PA and M+ (day 0) treatments exhibited significantly smaller P contents in the AMF (Figure 4). Although the hyphal content of P was significantly larger for the fungal compartment containing OP, the total amount of P accumulated in the hyphae was smaller, as there was significantly less AMF biomass. On average, the extraradical hyphae accumulated less P in the fungal compartment containing OP (0.03 mg P; 0.1%) and PA (0.015 mg P; 0.05%) as compared to the goethite-bound forms GOE-OP (2.39 mg P; 8%), and P for GOE-PA (1.22 mg P; 4.1%) (Figure 3). When these P amounts are summed up to the P incorporated by the plant, *R. irregularis* mobilised 9.15 mg P (30.5% of the initially added P) from the OP, followed by GOE-OP (4.17 mg P; 13.9%), PA (3.14 mg P; 10.5%), and GOE-PA (1.86 mg P; 6.2%) (Figure 3). Compared to that, P desorption from the goethite in water was 7.4% for GOE-OP and 1.2% for GOE-PA. These proportions corresponded to P amounts of 2.2 ± 0.25 mg in the GOE-OP treatment and 0.35 ± 0.008 mg P in the GOE-PA treatment, which likely were mobilised by desorption processes. Hence, the difference between the P incorporated in the plant tissues/AM mycelium and the P desorbed in water of the goethite-bound P sources, resulted in a net P mobilisation from the goethite complexes by the AMF between 5% (GOE-PA) and 6.5% (GOE-OP) of the initially added P.

Organic C contents (mg/g fungal compartment) show that the M- control treatment did not accumulate any C throughout the experiment. From day 7 to day 91, fungal compartments containing OP, PA and M+ control showed significantly smaller accumulation of OC compared to GOE-OP, GOE-PA, and GOE control. From day 7 to 77, the fungal compartment containing PA showed significantly larger OC contents than those with OP and control without P (M+) (Figure 5A). The same occurred for fungal compartments containing GOE-PA as compared to those with GOE-OP and GOE control (Figure 5A).

In case of cumulative CO_2 production (mg/g fungal compartment), the M- treatment, without mycorrhiza and without P, showed significantly smaller production than any other treatment (Figure 5B). Fungal compartments containing OP, PA and the M+ control showed significantly smaller cumulative CO_2 production from day 7 to 91 as compared to the production of GOE-OP, GOE-PA, and the GOE control. The cumulative CO_2 production showed no significant difference between those containing OP, PA and the M+ control. Throughout the experiment, the GOE control showed significantly larger cumulative CO_2 production values than any other treatment.

At days 77 and 91, when AM plants have reached their maximum P incorporation, the Total C/P was significantly higher for those AM plants that had access to GOE-OP, GOE-PA, and PA, as compared to OP (Figure 6). The P incorporated from GOE-OP showed larger PLFA 16:1ω5c/P ratios than the OP and PA treatments at days 77 and 91, while for GOE-PA this ratio was larger than for OP and PA at day 91 only (Figure 7A). The NLFA 16:1ω5c/P was significantly larger for both P sources bound to goethite in comparison to OP and PA (Figure 7B). For all P sources, the NLFA 16:1ω5c/PLFA 16:1ω5c ratio was above one, meaning that the PLFA and NLFA originated largely from AM fungi (Figure 7C). The NLFA 16:1ω5c/PLFA 16:1ω5c ratios increased for all AM plants accessing a P source in the fungal compartment during the period of P incorporation. The NLFA 16:1ω5c/PLFA 16:1ω5c ratio showed always significant larger values for the treatment offering GOE-OP as P source.

Discussion

Phosphorus Plant Uptake from Different Sources

We tested the role of AMF in taking up P from four different sources having a different accessibility. For this, a mesocosm was designed where exclusively the AM hyphae were involved in plant P acquisition. Our results indicate that all AM plants mycorrhized with *R. irregularis* incorporated P derived from inorganic (OP) or organic (PA) sources, added either in their free form or bound to goethite. Phosphorus from the different P forms was taken up in different amounts and kinetics via the mycorrhizal pathway. In contrast to other studies where roots and hyphae are only separated by a 20 µm nylon mesh (Argüello et al., 2016; Jakobsen et al., 2016; Sawers et al., 2017; Zhang et al., 2016), the hydrophobic polytetrafluoroethylene membrane used in our mesocosms avoided not only the direct P uptake by roots, but also the influence of root exudates and the diffusive transport from the fungal compartment to plant compartment, as well as the P absorption by the roots. This membrane type has been successfully used in other studies investigating on nitrogen transfer by AMF (Frey et al., 1998; Mäder et al., 1993, 2000), but not for P nutrition via the mycorrhizal pathway. The hydrophobic and selective nature of this barrier, however, is of paramount importance for separating the effects of the root and the fungus and quantifying the nutrient fluxes. These first results do thus not only give evidence of the importance for mycorrhiza in the uptake of P but also proof the advantage of hydrophobic polytetrafluoroethylene membranes (Figure 1).

Plant P incorporation appeared quickest in OP and PA treatments, followed one month later by the P forms bound to goethite. In addition, P from the freely accessible forms of OP and PA was incorporated to a greater extent (Figure 2). The faster plant P uptake in case of OP can be explained by the fact that an AM plant can absorb OP directly via the mycorrhizal pathway (Smith et al., 2003). Usually most of the studies on plant nutrition only consider inorganic phosphate—but not the organic P fraction—as biologically available, even though organic P represents the major part of the total soil P (Turner 2008). To be available for the plant, PA has to be hydrolyzed first by phytases, a group of phosphatases, which are either of plant or microbial origin (Baldwin et al., 2001, 2008; Javaid, 2009; Li et al., 1997). In our study we did not measure enzymatic activities, but it has been demonstrated that *R. irregularis* DAOM 197198 is able to secrete acid phosphatase, which contributes to the mineralization of P-bearing organic compounds in soil and thus increases the concentration of available inorganic P (Tisserant et al., 2012). Tarafdar and Marschner (1994a, b) reported the mobilisation of organic P sources for plant P uptake by exudation of fungal acid phosphatase in an experiment using *Glomus mosseae*. Accumulating evidence suggests that *Rhizophagus* species can hydrolyze organic P forms and the resultant inorganic P can be taken up and transported to host roots (Joner et al., 2000; Koide and Kabir, 2000; Sato et al., 2015). Utilization of organic P is thus assumed to contribute in a comparable manner to AM plant P nutrition as inorganic P (Feng et al., 2003), as we did not find significant differences among the amount of P mobilised from OP and PA.

We also did not find differences in the amount of P incorporated into the plant between the treatments offering GOE-OP and GOE-PA as P sources. Phosphorus from both P-goethite associations was incorporated by the mycorrhizal plant on average less and also later than free OP. Concerning OP, our results support earlier conclusions by Parfitt (1979), who provided the only available study addressing the mobilisation of OP adsorbed to goethite. They showed that *Lolium perenne* mycorrhized with *Glomus tenuis* desorbed phosphate from the goethite after 55 days. However, in this experiment there was no physical separation between the plant and hyphae. For that reason, Parfitt's results should be interpreted with caution, as desorption may be partially caused by the acidification of the rhizosphere mediated by root exudates containing an excess of protons, and not exclusively by the AMF (Hinsinger and Gilkes, 1996). To our knowledge, this is the first experiment proving that an AM plant incorporated P from PA bound to a secondary mineral. Noteworthy, the amounts of mobilised P were larger than the portion of most weakly sorbed PA, as determined in the separate desorption experiment. This finding suggests that AMF play an active role in the mobilisation of mineral-bound P. A possible mechanism used by AMF to desorb P from the surface of the goethite is through the release of exudates containing organic acids, as observed by Tawaraya et al. (2006). The delay to incorporate P from GOE-OP and GOE-PA associations may be due to two reasons: firstly, both P forms had to be desorbed from the goethite by means of organic acids; with the P diffusion being likely impaired in the goethite substrate. Secondly, in contrast to the desorbed OP, the released PA had still to be hydrolyzed through the action of phosphatases.

AMF not only supplied P to the plant, but also accumulated it into the hyphae grown in the fungal compartment. On the one hand, the P contents in the AM hyphae were larger for the treatments offering free P forms OP and PA, followed by those treatments offering GOE-OP and GOE-PA as P sources (Figure 4). On the other hand, the P stocks in the fungal biomass for OP and PA were significantly smaller than those determined for GOE-OP and GOE-PA (Figure 3). Thus, the P from the goethite-bound forms was evenly distributed between the fungus and the plant, while the non-bound forms were preferably incorporated into the plant. Our results point to a smaller P content inside the hyphae growing in the fungal compartments with the lees P accessible sources, GOE-OP and GOE-PA. This result would be in accordance with the observations made by Ezawa et al. (2002), who found that the P content in the hyphae appears to respond flexibly to P availability in the environment, varying its internal content proportionally.

R. irregularis did not transfer all of the mobilised P from the goethite sources to the plant and stored it within its hyphae, may be due to the fungus strategy to accumulate P under deficient conditions to keep a well-balanced homeostasis (Solaiman et al., 1999). Another possible interpretation of the P accumulation within the hyphae/spores in a broader sense, could be significant from the perspective of the AM plant in the common mycorrhizal network, where accumulated P indicates a likely support for the growth of a new mycelium and maybe even act as an ‘entrance fee’ for a new colonization (Hammer et al., 2011b; Olsson et al., 2008). Likewise, this accumulation of P would not be a futile waste of energy, due to the fact that P storage within microbial biomass may account for 1–10% (10–100 kg of P/ha) of the total soil

P (Richardson, 2001) and may become available to plants once the microbes die (Deubel and Merbach, 2005).

Carbon and Phosphorus Trading

No OC was detected in the fungal compartment for M- control treatment, while treatments containing PA and goethite-P associations showed larger OC contents as compared to those fungal compartments for OP and control M+ (Figure 5A). AMF tends to develop more readily under nutrient-poor conditions than under nutrient-rich or heavily P-fertilized conditions (Bryla and Eissenstat, 2005; Olsson et al., 2010), as would be the case for those fungal compartments where there was GOE, PA and sources of goethite-P. Before any P was incorporated between days 0 to 35, the GOE-OP, GOE-PA and GOE treatments exhibited significantly higher OC contents. The data suggests that OC increased more rapidly in those fungal compartments containing goethite compared to fungal compartments with OP or PA. Supposedly, this is due to a greater investment of photoassimilates via the AMF to mobilise less accessible forms before and during the P acquisition. The organic substances exuded by the AMF may be also well stabilized against fast microbial decomposition, as goethite effectively binds organic molecules via ligand exchange below its point of zero charge (Kaiser and Guggenberger, 2007).

Along with the larger OC contents in the fungal compartment of the AM plants, we detected a larger CO_2 production at all sampling points in any of the AM plant treatments, especially those where goethite was present, as for the M- treatment (Figure 5B). The total CO_2 production might have been a bit overestimated as we also measured a small CO_2 production in the M- treatment, which was likely caused by gas permeability of the two membranes between the plant and the fungal compartment. We also cannot exclude the presence of root CO_2 production in treatments with AM plants. However, similar mesocosm systems were used to indirectly estimate CO_2 production of extraradical mycelium (Heinemeyer et al., 2006; Karasawa et al., 2012; Nottingham et al., 2010), and different calculations were applied to estimate the contribution of AM extraradical mycelium, such as the difference in total CO_2 emission between AM and non-AM fungal compartments (Karasawa et al., 2012). We did not indirectly estimate the CO_2 production of the extraradical mycelium as a variable soil nutrient availability is known to demand different amounts of root and respiratory products (Atkin et al., 2009). In line with Atkin et al. (2009), we could show that AM tomato plants were associated with larger CO_2 rates in the fungal compartment compared with M- plants and increased soil CO_2 release. The nature of the respired CO_2 at the fungal compartment, is the sum of the autotrophic and the heterotrophic respirations. The autotrophic respiration comprises the fraction derived from current photosynthates (Olsson et al., 2005), and in our case includes the respiration by mycorrhizal fungi, and likely other microorganisms using C that has been recently fixed by plant photosynthesis, since we inoculated the plants with *R. irregularis* carrying the microorganisms naturally associated with its hyphosphere. The heterotrophic respiration is due to the decomposition of OC (Kuzyakov and Gavrichkova, 2010) made up mostly of dead hyphae and bacteria, mediated by the microbes present at the fungal compartment. Taking together the results of OC content and CO_2 production in the fungal compartment, we can assume that treatments containing less accessible phosphorus sources showed higher C investments into autotrophic and heterotrophic metabolic processes in the fungal compartment, before and during the incorporation of P in the AM plant. Consequently, the Total C/P ratio was significantly larger in those fungal compartments containing free PA and both P sources bound to goethite (Figure 6). The total C as the sum of total OC and cumulative CO_2 enabled to account for most of the C exclusively carried by the AMF into the fungal compartment. The results point to a greater investment of plant photoassimilates in the fungal compartments compared to the free OP treatment and controls. Similarly, a more active metabolism for the less accessible P sources was indicated by a larger CO_2 production for the same amount of P mobilised from PA, GOE-OP and GOE-PA compared to OP. Respiratory energy is required by the microbiont for constructing new intraradical and extraradical fungal tissue (including reproductive structures), for maintenance and repair of existing fungal tissue and for cellular processes in the fungal tissue associated with the absorption and transfer of nutrients to the host (Bryla and Eissenstat, 2005). The photoassimilate investment into respiration (fast) or structural (slow) C pools in soil has been reported frequently before (Zhu and Miller, 2003), but were not related to a direct nutrient allocation mechanism as in our case. Carbon fractions with slow and fast turnover directly translate into residence time of C in soil, thus to the C sequestration potential of different functional traits of AM concerning their mobilisation of nutrients. Staddon (2003) suggested AM hyphae do not contribute substantially to C sequestration in soil. In contrast, our results point toward larger C sequestration rates and metabolic activity in the less accessible sources, being consistent with the general line of argumentation of Turner (2008). This energy investment into fungal compartments with less accessible P sources, taken together with more structural C contents found in these compartments clearly show that AM fungi are able to adjust to the given requirements of mobilising their nutritional sources.

Growth of *R. irregularis* under Variable Phosphorus Availability

The PLFA 16:1ω5c /P and NLFA 16:1ω5c/P showed a significant larger amount of both fatty acids accumulated into the fungal compartment relative to P uptake, in those fungal compartments containing GOE-OP and GOE-PA compared to OP and PA (Figure 7A and 7B). The fungal lipids extracted from the fungal compartment indicate a different growth strategy to mobilise P from the goethite-bound compounds compared to the free forms. The AMF grown in those fungal compartment containing GOE-OP and PA accumulated two and three orders of magnitude more PLFA and NLFA, respectively, compared to OP and PA. This observation confirms a larger C investment in energy storage and mycelium per P incorporated into the AM plant. *R. irregularis* grown in the fungal compartment containing GOE-OP accumulated significant more NLFA, as indicated by the ratio NLFA 16:1ω5c/PLFA 16:1ω5c, meaning that the AMF stored energy in the form of neutral lipids before and during the P mobilisation, compared to GOE-PA and the free P forms (Figure 7C). One potential explanation for the more pronounced development of fungal infrastructure in the presence of P forms bound to goethite is the need to establish a sufficient extraradical hyphae of *R. irregularis* to mobilise and transport P more efficiently to the plant (Abbott and Robson, 1982; Joner et al., 2000; Smith et al., 2003). According to Bryla and Eissenstat (2005), mycorrhizas tend to be favored under nutrient-poor conditions, as in case of the goethite-P treatments. The reduced hyphal growth in both fungal compartment containing OP and PA with a high P content might indicate that the roots may reduce the C flow to the fungus under larger P availability (Olsson et al., 1997, 2002). It has been suggested that a reduced carbohydrate allocation would be a regulatory saving mechanism by the plant host where there is an excess of phosphate ion (Konvalinková et al., 2017; Torres de los Santos et al., 2016). Bååth (2003) showed the NLFA 16:1ω5c/PLFA 16:1ω5c is a good indicator to express preferential C allocation to storage products, when comparing different nutritional scenarios. Our results corroborate this observation and suggest that the preferential energy storage in the form of neutral lipid could be used to support the extensive growth of *R. irregularis* mycelium in the goethite systems.

The plant is often considered as being in control of the symbiosis, and this “phytocentric” view is supported by the idea that AM symbiosis is a strategy that could be suppressed by the plants at sufficient P supply. But in natural ecosystems, instead of luxurious P availability, suppression of AM colonization by the plant is not the normal situation (Smith and Smith, 2012), as plants have evolved diverse adaptations to acquire different forms of soil P (Ceulemans et al., 2017). Hammer et al. (2011) suggest a fungal control mechanism of the P transfer to the plant, and that the C-P exchange between the symbionts is closely linked. Our results, showing a different content and partitioning of C into NLFA and PLFA 16:1ω5c for those *R. irregularis* grown in treatments with mineral-bound P sources, corroborate that point. This more “mycocentric” view of symbiosis explains the evolutionarily stable mutualistic relationship between these symbionts. If AMF are able to reduce the delivery of P to plants with a limited supply of C (Kiers et al., 2011), they possess an important function to actively increase their fitness by choosing the best plant partner, which in turn could increase selection pressure on the plant species within a community (Hammer et al., 2011b). Under our experimental conditions with forcing plants to cover their P supply from a single source, we observed different trading costs between C for P, distinct P acquisition strategies resulting in differing C costs for the AM plant. This observation is in agreement with Turner’s idea (2008), where the different P compounds are classified by the investment they make to access phosphate ions, which in term is dictated by the biological availability of the given source.

Conclusions

Our study provided evidence that AM plants are able to mobilise P sources differing in their accessibility. This happened exclusively via the mycorrhizal pathway at contrasting kinetics and accumulation rates, driven by the amount of photoassimilates needed for the mobilisation of the P sources. The mobilised P was redistributed in different amounts into the fungus itself and the plant, which again was influenced by the P species. Both P sources adsorbed to goethite (GOE-OP, GOE-PA) facilitated a greater investment of the AM plant into the production of fungal vegetative structures than in case of the free OP and PA. Larger contents of PLFA 16:1ω5c and NLFA 16:1ω5c were observed especially in systems with goethite-associated P, suggesting the accumulation of significantly larger amounts of extraradical hyphae. Further, the lipid accumulation in form of PLFA 16:1ω5c and NLFA 16:1ω5c suggests different growth strategies to mobilise P from the goethite-bound compounds. This is also mirrored by the larger C investment per P incorporated, as compared to OP and PA. Our data also suggest that the C investment by mycorrhized plants into P acquisition from differently available P sources has a direct effect on the amount of C accumulating in soils. By identifying even better suitable AMF strains able to mobilise even hard-to-exploit sources, nutrient efficiency in agricultural systems might be promoted and in this way reduce as much as possible the dependence on P fertilizers.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

We want to thank the German Federal Ministry of Education and Research for the funding of this project in the framework of the DFG-RTG 1798 “Signaling at the Plant-Soil Interface” as well as the DFG priority program SPP 1685 “Forest Strategies for limited Phosphorus Resources”.

Acknowledgments

The authors are thankful for the great help and guidance received by Silke Bokeloh, Elke Eichmann-Prusch, Ulrike Pieper, Heike Steffen, and Michael Klatt.

References

Aarle, I. M. Van, and Olsson, P. A. (2003). Fungal Lipid Accumulation and Development of Mycelial Structures by Two Arbuscular Mycorrhizal Fungi Fungal Lipid Accumulation and Development of Mycelial Structures by Two Arbuscular Mycorrhizal Fungi. *Appl. Environ. Microbiol.* 69, 6762–6767. doi:10.1128/AEM.69.11.6762.

Abbott, L. K., and Robson, A. D. (1982). The role of vesicular arbuscular mycorrhizal fungi in agriculture and the selection of fungi for inoculation. *Aust. J. Agric. Res.* 33, 389–408. doi:10.1071/AR9820389.

Argüello, A., O’Brien, M. J., van der Heijden, M. G. A., Wiemken, A., Schmid, B., and Niklaus, P. A. (2016). Options of partners improve carbon for phosphorus trade in the arbuscular mycorrhizal mutualism. *Ecol. Lett.* 19, 648–656. doi:10.1111/ele.12601.

Atkin, O. K., Sherlock, D., Fitter, A. H., Jarvis, S., Hughes, J. K., Campbell, C., et al. (2009). Temperature dependence of respiration in roots colonized by arbuscular mycorrhizal fungi. *New Phytol.* 182, 188–199. doi:10.1111/j.1469-8137.2008.02727.x.

Bååth, E. (2003). The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. *Microb. Ecol.* 45, 373–383. doi:10.1007/s00248-003-2002-y.

Bago, B., Zipfel, W., Williams, R. M., Jun, J., Arreola, R., Lammers, P. J., et al. (2002).

Translocation and utilization of fungal storage lipid in the arbuscular mycorrhizal symbiosis. *Plant Physiol.* 128, 108–124. doi:10.1104/pp.010466.

Baldwin, J. C., Karthikeyan, A. S., Cao, A., and Raghothama, K. G. (2008). Biochemical and molecular analysis of LePS2;1: A phosphate starvation induced protein phosphatase gene from tomato. *Planta* 228, 273–280. doi:10.1007/s00425-008-0736-y.

Baldwin, J. C., Karthikeyan, A. S., and Raghothama, K. G. (2001). LEPS2, a phosphorus starvation-induced novel acid phosphatase from tomato. *Plant Physiol.* 125, 728–37. doi:10.1104/pp.125.2.728.

Becquer, A., Trap, J., Irshad, U., Ali, M. A., and Claude, P. (2014). From soil to plant, the journey of P through trophic relationships and ectomycorrhizal association. *Front. Plant Sci.* 5, 548. doi:10.3389/fpls.2014.00548.

Bischoff, N., Mikutta, R., Shibistova, O., Puzanov, A., Reichert, E., Silanteva, M., et al. (2016). Land-use change under different climatic conditions: Consequences for organic matter and microbial communities in Siberian steppe soils. *Agric. Ecosyst. Environ.* 235, 253–264. doi:10.1016/j.agee.2016.10.022.

Bolan, N. S., Robson, A. D., and Barrow, N. J. (1987). Effects of vesicular-arbuscular mycorrhiza on the availability of iron phosphates to plants. *Plant Soil* 99, 401–410. doi:10.1007/BF02370885.

Bolan, N. S. S., Robson, A. D. D., Barrow, N. J. J., and Aylmore, L. A. G. A. G. (1984). Specific activity of phosphorus in mycorrhizal and non-mycorrhizal plants in relation to the availability of phosphorus to plants. *Soil Biol. Biochem.* 16, 299–304. doi:10.1016/0038-0717(84)90023-3.

Bravo, A., Brands, M., Wewer, V., Dörmann, P., and Harrison, M. J. (2017). Arbuscular mycorrhiza-specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote development of arbuscular mycorrhiza. *New Phytol.* 214, 1631–1645. doi:10.1111/nph.14533.

Brundrett, M. (1996). *Working with mycorrhizas in forestry and agriculture*. Canberra: Australian Centre for International Agricultural Research.

Bryla, D. R., and Eissenstat, D. M. (2005). “Respiratory Costs of Mycorrhizal Associations,” in *Plant Respiration* (Berlin/Heidelberg: Springer-Verlag), 207–219. doi:10.1007/1-4020-

3589-6_12.

Cairney, J. W. G. (2000). Evolution of mycorrhiza systems. *Naturwissenschaften* 87, 467–475. doi:10.1007/s001140050762.

Calonne, M., Fontaine, J., Debiane, D., Laruelle, F., Grandmougin-ferjani, A., and Sahraoui, A. L. (2010). Propiconazole Toxicity on the Non-Target Organism , the Arbuscular Mycorrhizal Fungus, Glomus irregulare. *Fungicides*, 325–346.

Cardoso, I. M., Boddington, C. L., Janssen, B. H., Oenema, O., and Kuyper, T. W. (2006). Differential Access to Phosphorus Pools of an Oxisol by Mycorrhizal and Nonmycorrhizal Maize. *Commun. Soil Sci. Plant Anal.* 37, 1537–1551. doi:10.1080/00103620600710074.

Cavagnaro, T. R., Langley, A. J., Jackson, L. E., Smukler, S. M., and Koch, G. W. (2008). Growth, nutrition, and soil respiration of a mycorrhiza-defective tomato mutant and its mycorrhizal wild-type progenitor. *Funct. Plant Biol.* 35, 228. doi:10.1071/FP07281.

Ceulemans, T., Bodé, S., Bollyn, J., Harpole, S., Coorevits, K., Peeters, G., et al. (2017). Phosphorus resource partitioning shapes phosphorus acquisition and plant species abundance in grasslands. *Nat. Plants* 3, 16224. doi:10.1038/nplants.2016.224.

Cozzolino, V., Di Meo, V., Monda, H., Spaccini, R., and Piccolo, A. (2016). The molecular characteristics of compost affect plant growth, arbuscular mycorrhizal fungi, and soil microbial community composition. *Biol. Fertil. Soils* 52, 15–29. doi:10.1007/s00374-015-1046-8.

Daubois, L., Beaudet, D., Hijri, M., and De La Providencia, I. (2016). Independent mitochondrial and nuclear exchanges arising in Rhizophagus irregularis crossed-isolates support the presence of a mitochondrial segregation mechanism Ecological and evolutionary microbiology. *BMC Microbiol.* 16, 11. doi:10.1186/s12866-016-0627-5.

Deubel, A., and Merbach, W. (2005). "Influence of Microorganisms on Phosphorus Bioavailability in Soils," in *Microorganisms in Soils: Roles in Genesis and Functions* (Berlin/Heidelberg: Springer-Verlag), 177–191. doi:10.1007/3-540-26609-7_9.

Ezawa, T., Smith, S. E., and Smith, F. A. P. (2002). P metabolism and transport in AM fungi. *Plant Soil* 244, 221–30.

Feng, G., Song, Y. . C., Li, X. . L., and Christie, P. (2003). Contribution of arbuscular mycorrhizal fungi to utilization of organic sources of phosphorus by red clover in a calcareous soil. *Appl. Soil Ecol.* 22, 139–148. doi:10.1016/S0929-1393(02)00133-6.

Fitter, A. H., Graves, J. D., Watkins, N. K., Robinson, D., and Scrimgeour, C. (1998). Carbon transfer between plants and its control in networks of arbuscular mycorrhizas. *Funct. Ecol.* 12, 406–412. doi:10.1046/j.1365-2435.1998.00206.x.

Frey, B., Brunner, I., Christie, P., Wiemken, A., and Mäder, P. (1998). “The Use of Polytetrafluoroethylene (PTFE) Hydrophobic Membranes to Study Transport of 15N by Mycorrhizal Hyphae,” in *Mycorrhiza Manual* (Berlin, Heidelberg: Springer Berlin Heidelberg), 151–158. doi:10.1007/978-3-642-60268-9_10.

Frossard, E., Achat, D. L., Bernasconi, S. M., Bünemann, E. K., Fardeau, J.-C., Jansa, J., et al. (2011). “The Use of Tracers to Investigate Phosphate Cycling in Soil–Plant Systems,” in (Springer Berlin Heidelberg), 59–91. doi:10.1007/978-3-642-15271-9_3.

Frostegård, Å., Tunlid, A., and Bååth, E. (1991). Microbial biomass measured as total lipid phosphate in soils of different organic content. *J. Microbiol. Methods* 14, 151–163. doi:10.1016/0167-7012(91)90018-L.

García, G. H. G. C. A. (2000). Behaviour of arbuscular-mycorrhizal fungi on Vigna luteola growth and its effect on the exchangeable (32 P) phosphorus of soil. *Biol. Fertil. Soils* 31, 232–236. doi:10.1007/s003740050650.

Gentsch, N., Wild, B., Mikutta, R., Čapek, P., Diáková, K., Schrumpf, M., et al. (2018). Temperature response of permafrost soil carbon is attenuated by mineral protection. *Glob. Chang. Biol.* 24, 3401–3415. doi:10.1111/gcb.14316.

George, T. S., Hinsinger, P., and Turner, B. L. (2016). Phosphorus in soils and plants: facing phosphorus scarcity. *Plant Soil* 401, 1–6. doi:10.1007/s11104-016-2846-9.

Gilbert, N. (2009). Environment: The disappearing nutrient. *Nature* 461, 716–8. doi:10.1038/461716a.

Giles, C. D., Richardson, A. E., Druschel, G. K., and Hill, J. E. (2012). Organic anion-driven solubilization of precipitated and sorbed phytate improves hydrolysis by phytases and bioavailability to Nicotiana tabacum. *Soil Sci.* 177, 591–598. doi:10.1097/SS.0b013e318272f83f.

Giovannetti, M., Avio, L., Barale, R., Ceccarelli, N., Cristofani, R., Iezzi, A., et al. (2012). Nutraceutical value and safety of tomato fruits produced by mycorrhizal plants. *Br. J. Nutr.*

107, 242–51. doi:10.1017/S000711451100290X.

Green, H., Larsen, J., Olsson, P. A., Jensen, D. F., and Jakobsen, I. (1999). Suppression of the biocontrol agent Trichoderma harzianum by mycelium of the arbuscular mycorrhizal fungus Glomus intraradices in root-free soil. *Appl. Environ. Microbiol.* 65, 1428–1434.

Hammer, E. C., Nasr, H., Pallon, J., Olsson, P. A., and Wallander, H. (2011a). Elemental composition of arbuscular mycorrhizal fungi at high salinity. *Mycorrhiza* 21, 117–129. doi:10.1007/s00572-010-0316-4.

Hammer, E. C., Pallon, J., Wallander, H., and Olsson, P. A. (2011b). Tit for tat? A mycorrhizal fungus accumulates phosphorus under low plant carbon availability. *FEMS Microbiol. Ecol.* 76, 236–244. doi:10.1111/j.1574-6941.2011.01043.x.

He, Z., and Zhu, J. U. N. (1998). Microbial utilization and transformation of phosphate adsorbed by variable charge minerals. *Soil Biol. Biochem.* 30, 917–923. doi:10.1016/S0038-0717(97)00188-0.

Heinemeyer, A., Ineson, P., Ostle, N., and Fitter, A. H. (2006). Respiration of the external mycelium in the arbuscular mycorrhizal symbiosis shows strong dependence on recent photosynthates and acclimation to temperature. *New Phytol.* 171, 159–170. doi:10.1111/j.1469-8137.2006.01730.x.

Hewitt, E. J. (1966). *Sand and water culture methods used in the study of plant nutrition*. Technichal. East Malling , Maidstone, Kent.: University of Bristol Agricultural and Horticultural Research Station, Long Ashton, Bristol.

Hinsinger, P., and Gilkes, R. J. (1996). Mobilisation of phosphate from phosphate rock and alumina-sorbed phosphate by the roots of ryegrass and clover as related to rhizosphere pH. *Eur. J. Soil Sci.* 47, 533–544. doi:10.1111/j.1365-2389.1996.tb01853.x.

IBM Corporation (2016). IBM SPSS Statistics for Windows, Version 24.0. *2016*.

Jakobsen, I., Smith, S. E., Smith, F. A., Watts-Williams, S. J., Clausen, S. S., and Grønlund, M. (2016). Plant growth responses to elevated atmospheric CO 2 are increased by phosphorus sufficiency but not by arbuscular mycorrhizas. *J. Exp. Bot.* 67, 6173–6186. doi:10.1093/jxb/erw383.

Javaid, A. (2009). Arbuscular Mycorrhizal Mediated Nutrition in Plants. *J. Plant Nutr.* 32, 1595–1618. doi:10.1080/01904160903150875.

Javot, H., Pumplin, N., and Harrison, M. J. (2007). Phosphate in the arbuscular mycorrhizal symbiosis: transport properties and regulatory roles. *Plant. Cell Environ.* 30, 310–22. doi:10.1111/j.1365-3040.2006.01617.x.

Johansen, A., Finlay, R. D., and Olsson, P. A. (1996). Nitrogen metabolism of external hyphae of the arbuscular mycorrhizal fungus Glomus intraradices. *New Phytol.* 133, 705–712. doi:10.1111/j.1469-8137.1996.tb01939.x.

Johnson, N. C. (2010). Resource stoichiometry elucidates the structure and function of arbuscular mycorrhizas across scales. *New Phytol.* 185, 631–647. doi:10.1111/j.1469-8137.2009.03110.x.

Joner, E. J., Ravnskov, S., and Jakobsen, I. (2000). Arbuscular mycorrhizal phosphate transport under monoxenic conditions using radio-labelled inorganic and organic phosphate. *Biotechnol. Lett.* 22, 1705–1708. doi:10.1023/A:1005684031296.

Kaiser, K., and Guggenberger, G. (2007). Sorptive stabilization of organic matter by microporous goethite: Sorption into small pores vs. surface complexation. *Eur. J. Soil Sci.* 58, 45–59. doi:10.1111/j.1365-2389.2006.00799.x.

Karasawa, T., Hodge, A., and Fitter, A. H. (2012). Growth, respiration and nutrient acquisition by the arbuscular mycorrhizal fungus Glomus mosseae and its host plant Plantago lanceolata in cooled soil. *Plant, Cell Environ.* 35, 819–828. doi:10.1111/j.1365-3040.2011.02455.x.

Keymer, A., Pimprikar, P., Wewer, V., Huber, C., Brands, M., Bucerius, S. L., et al. (2017). Lipid transfer from plants to arbuscular mycorrhiza fungi. *Elife* 6. doi:10.7554/eLife.29107.

Kiers, E. T., Duhamel, M., Beesetty, Y., Mensah, J. A., Franken, O., Verbruggen, E., et al. (2011). Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *Science (80-.).* 333, 880–882. doi:10.1126/science.1208473.

Köhl, L., Lukasiewicz, C. E., and Van der Heijden, M. G. A. (2016). Establishment and effectiveness of inoculated arbuscular mycorrhizal fungi in agricultural soils. *Plant Cell Environ.* 39, 136–146. doi:10.1111/pce.12600.

Koide, R. T., and Kabir, Z. (2000). Extraradical hyphae of the mycorrhizal fungus Glomus intraradices can hydrolyse inorganic phosphate. *New Phytol.* 148, 511–517.

doi:10.1046/j.1469-8137.2000.00776.x.

Konvalinková, T., Püschel, D., Řezáčová, V., Gryndlerová, H., and Jansa, J. (2017). Carbon flow from plant to arbuscular mycorrhizal fungi is reduced under phosphorus fertilization. *Plant Soil* 419, 1–15. doi:10.1007/s11104-017-3350-6.

Krüger, M., Krüger, C., Walker, C., Stockinger, H., Schüßler, A., Kr??ger, M., et al. (2012). Phylogenetic reference data for systematics and phylotaxonomy of arbuscular mycorrhizal fungi from phylum to species level. *New Phytol.* 193, 970–984. doi:10.1111/j.1469-8137.2011.03962.x.

Kuntz, L. B. (2013). Wick Irrigation Systems for Subsistence Farming. Available at: https://dspace.mit.edu/handle/1721.1/83726 [Accessed October 21, 2018].

Kuzyakov, Y., and Gavrichkova, O. (2010). Time lag between photosynthesis and carbon dioxide efflux from soil: A review of mechanisms and controls. *Glob. Chang. Biol.* 16, 3386–3406. doi:10.1111/j.1365-2486.2010.02179.x.

Lambers, H., Martinoia, E., and Renton, M. (2015). Plant adaptations to severely phosphorus-impoverished soils. *Curr. Opin. Plant Biol.* 25, 23–31. doi:10.1016/j.pbi.2015.04.002.

Larsen, J., Olsson, P. A., and Jakobsen, I. (1998). The use of fatty acid signatures to study mycelial interactions between the arbuscular mycorrhizal fungus Glomus intraradices and the saprotrophic fungus Fusarium culmorum in root-free soil. *Mycol. Res.* 102, 1491–1496. doi:10.1017/S0953756298006558.

Li, M., Osaki, M., Honma, M., and Tadano, T. (1997). Purification and characterization of phytase induced in tomato roots under phosphorus-deficient conditions. *Soil Sci. Plant Nutr.* 43, 179–190. doi:10.1080/00380768.1997.10414726.

Luginbuehl, L. H., Menard, G. N., Kurup, S., Van Erp, H., Radhakrishnan, G. V, Breakspear, A., et al. (2017). Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant. *Science* 356, 1175–1178. doi:10.1126/science.aan0081.

Mäder, P., Vierheilig, H., Alt, M., and Wiemken, A. (1993). Boundaries between soil compartments formed by microporous hydrophobic membranes (GORE-TEXR) can be crossed by vesicular-arbuscular mycorrizal fungi but not by ions in the soil solution. *Plant Soil* 152, 201–206. doi:10.1007/BF00029089.

Mäder, P., Vierheilig, H., Streitwolf-Engel, R., Boller, T., Frey, B., Christie, P., et al. (2000).

Transport of 15N from a soil compartment separated by a polytetrafluoroethylene membrane to plant roots via the hyphae of arbuscular mycorrhizal fungi. *New Phytol.* 146, 155–161. doi:10.1046/j.1469-8137.2000.00615.x.

Marschner, P. (2007). “Soil Microbial Community Structure and Function Assessed by FAME, PLFA and DGGE — Advantages and Limitations,” in *Advanced Techniques in Soil Microbiology* (Berlin, Heidelberg: Springer Berlin Heidelberg), 181–200. doi:10.1007/978-3-540-70865-0.

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., and Swan, J. A. (1990). A New Method which Gives an Objective Measure of Colonization of Roots by Vesicular-Arbuscular Mycorrhizal Fungi. *New Phytol.* 115, 495–501. doi:10.1111/j.1469-8137.1990.tb00476.x.

Mengel, K., and Kirkby, E. A. (2001). *Principles of Plant Nutrition.* , eds. K. Mengel, E. A. Kirkby, H. Kosegarten, and T. Appel Dordrecht: Springer Netherlands doi:10.1097/00010694-198407000-00012.

Nagy, R., Karandashov, V., Chague, V., Kalinkevich, K., Tamasloukht, M., Xu, G., et al. (2005). The characterization of novel mycorrhiza-specific phosphate transporters from Lycopersicon esculentum and Solanum tuberosum uncovers functional redundancy in symbiotic phosphate transport in solanaceous species. *Plant J.* 42, 236–50. doi:10.1111/j.1365-313X.2005.02364.x.

Nash, D. M., Haygarth, P. M., Turner, B. L., Condron, L. M., McDowell, R. W., Richardson, A. E., et al. (2014). Using organic phosphorus to sustain pasture productivity: A perspective. *Geoderma* 221–222, 11–19. doi:10.1016/j.geoderma.2013.12.004.

Nottingham, A. T., Turner, B. L., Winter, K., van der Heijden, M. G. A., and Tanner, E. V. J. (2010). Arbuscular mycorrhizal mycelial respiration in a moist tropical forest. *New Phytol.* 186, 957–967. doi:10.1111/j.1469-8137.2010.03226.x.

Oberson, A., Friesen, D. K., Rao, I. M., Bühler, S., Frossard, E., Buhler, S., et al. (2001). Phosphorus transformations in an Oxisol under contrasting land-use systems: The role of the soil microbial biomass. in *Plant and Soil* (Kluwer Academic Publishers), 197–210. doi:10.1023/A:1013301716913.

Ognalaga, M., Frossard, E., and Thomas, F. (1994). Glucose-1-phosphate and myo-inositol hexaphosphate adsorption mechanisms on goethite. *Soil Sci. Soc. Am. J.* 58, 332–337.

doi:10.2136/sssaj1994.03615995005800020011x.

Olsson, P. A. (1999). Signature fatty acids provide tools for determination of the distribution and interactions of mycorrhizal fungi in soil. *FEMS Microbiol. Ecol.* 29, 303–310. doi:10.1016/S0168-6496(99)00021-5.

Olsson, P. A., Bååth, E., and Jakobsen, I. (1997). Phosphorus effects on the mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by analysis of fatty acid signatures. *Appl. Environ. Microbiol.* 63, 3531–3538.

Olsson, P. A., Hammer, E. C., Wallander, H., and Pallon, J. (2008). Phosphorus Availability Influences Elemental Uptake in the Mycorrhizal Fungus Glomus intraradices, as Revealed by Particle-Induced X-Ray Emission Analysis. *Appl. Environ. Microbiol.* 74, 4144–4148. doi:10.1128/AEM.00376-08.

Olsson, P. A., and Johansen, A. (2000). Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. *Mycol. Res.* 104, 429–434. doi:10.1017/S0953756299001410.

Olsson, P. A., and Johnson, N. C. (2005). Tracking carbon from the atmosphere to the rhizosphere. *Ecol. Lett.* 8, 1264–1270. doi:10.1111/j.1461-0248.2005.00831.x.

Olsson, P. A., Rahm, J., and Aliasgharzad, N. (2010). Carbon dynamics in mycorrhizal symbioses is linked to carbon costs and phosphorus benefits. *FEMS Microbiol. Ecol.* 72, 125–131. doi:10.1111/j.1574-6941.2009.00833.x.

Olsson, P. A., van Aarle, I. M., Allaway, W. G., Ashford, A. E. A. E., and Rouhier, H. (2002). Phosphorus Effects on Metabolic Processes in Monoxenic Arbuscular Mycorrhiza Cultures. *Plant Physiol.* 130, 1162–1171. doi:10.1104/pp.009639.

Olsson, P. A., and Wilhelmsson, P. (2000). The growth of external AM fungal mycelium in sand dunes and in experimental systems. *Plant Soil* 226, 161–169. doi:10.1023/A:1026565314345.

Olsson, P., Linder, S., Giesler, R., and Högberg, P. (2005). Fertilization of boreal forest reduces both autotrophic and heterotrophic soil respiration. *Glob. Chang. Biol.* 11, 1745–1753. doi:10.1111/j.1365-2486.2005.001033.x.

Osorio, N. W., and Habte, M. (2001). Synergistic Influence of an Arbuscular Mycorrhizal Fungus and a P Solubilizing Fungus on Growth and P Uptake of Leucaena leucocephala in an Oxisol. *Arid L. Res. Manag.* 15, 263–274. doi:10.1080/15324980152119810.

Parfitt, R. L. (1979). The availability of P from phosphate-goethite bridging complexes. Desorption and uptake by ryegrass. *Plant Soil* 53, 55–65. doi:10.1007/BF02181879.

Pfeffer, P. E., Douds, D. D., Bücking, H., Schwartz, D. P., and Shachar-Hill, Y. (2004). The fungus does not transfer carbon to or between roots in an arbuscular mycorrhizal symbiosis. *New Phytol.* 163, 617–627. doi:10.1111/j.1469-8137.2004.01152.x.

Read, D. J. (1991). "Mycorrhizas in ecosystems - Nature's response to the 'Law of the minimum,'" in *Frontiers in Mycology*, ed. D. L. Hawksworth (Regensburg, Gemerany: CAB International), 101–130.

Richardson, A. E. (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Aust. J. Plant Physiol.* 28, 897–906. doi:10.1071/PP01093.

Richardson, A. E., Hocking, P. J., Simpson, R. J., and George, T. S. (2009). Plant mechanisms to optimise access to soil phosphorus. *Crop Pasture Sci.* 60, 124–143. doi:10.1071/CP07125.

Rinnan, R., and Bååth, E. (2009). Differential utilization of carbon substrates by bacteria and fungi in tundra soil. *Appl. Environ. Microbiol.* 75, 3611–3620. doi:10.1128/AEM.02865-08.

Rodrigues, M., Pavinato, P. S., Withers, P. J. A., Teles, A. P. B., and Herrera, W. F. B. (2016). Legacy phosphorus and no tillage agriculture in tropical oxisols of the Brazilian savanna. *Sci. Total Environ.* 542, 1050–1061. doi:10.1016/j.scitotenv.2015.08.118.

Rowe, H., Withers, P. J. A., Baas, P., Chan, N. I., Doody, D., Holiman, J., et al. (2016). Integrating legacy soil phosphorus into sustainable nutrient management strategies for future food, bioenergy and water security. *Nutr. Cycl. Agroecosystems* 104, 393–412. doi:10.1007/s10705-015-9726-1.

Rychter, A., Rao, I., and Cardoso, J. (2016). "Role of Phosphorus in Photosynthetic Carbon Assimilation and Partitioning," in *Handbook of Photosynthesis*, 603–625. doi:10.1201/b19498-45.

Sato, T., Ezawa, T., Cheng, W., and Tawaraya, K. (2015). Release of acid phosphatase from extraradical hyphae of arbuscular mycorrhizal fungus Rhizophagus clarus. *Soil Sci. Plant Nutr.* 61, 269–274. doi:10.1080/00380768.2014.993298.

Sawers, R. J. H., Svane, S. F., Quan, C., Grønlund, M., Wozniak, B., Gebreselassie, M. N., et al. (2017). Phosphorus acquisition efficiency in arbuscular mycorrhizal maize is correlated with the abundance of root-external hyphae and the accumulation of transcripts encoding PHT1 phosphate transporters. *New Phytol.* 214, 632–643. doi:10.1111/nph.14403.

Schaarschmidt, S., Roitsch, T., and Hause, B. (2006). Arbuscular mycorrhiza induces gene expression of the apoplastic invertase LIN6 in tomato (Lycopersicon esculentum) roots. *J. Exp. Bot.* 57, 4015–4023. doi:10.1093/jxb/erl172.

Slavikova, R., Püschel, D., Janouskova, M., Hujslova, M., Konvalinkova, T., Gryndlerova, H., et al. (2016). Monitoring CO2 emissions to gain a dynamic view of carbon allocation to arbuscular mycorrhizal fungi. *Mycorrhiza* 27, 1–17. doi:10.1007/s00572-016-0731-2.

Smith, F. A., and Smith, S. E. (2011). What is the significance of the arbuscular mycorrhizal colonisation of many economically important crop plants? *Plant Soil* 348, 63–79. doi:10.1007/s11104-011-0865-0.

Smith, S. E. S. E., Read, D. J., ., Kiers, E. T., Duhamel, M., Beesetty, Y., et al. (2008). *Mycorrhizal Symbiosis*. 3^{a}. Academic Press doi:10.1097/00010694-198403000-00011.

Smith, S. E., and Smith, F. A. (2012). Fresh perspectives on the roles of arbuscular mycorrhizal fungi in plant nutrition and growth. *Mycologia* 104, 1–13. doi:10.3852/11-229.

Smith, S. E., Smith, F. A., and Jakobsen, I. (2003). Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiol.* 133, 16–20. doi:10.1104/pp.103.024380.

Solaiman, M. Z., Ezawa, T., Kojima, T., and Saito, M. (1999). Polyphosphates in intraradical and extraradical hyphae of an arbuscular mycorrhizal fungus, Gigaspora margarita. *Appl. Environ. Microbiol.* 65, 5604–5606.

Son, J. E., Oh, M. M., Lu, Y. J., Kim, K. S., and Giacomelli, G. A. (2006). Nutrient-flow wick culture system for potted plant production: System characteristics and plant growth. *Sci. Hortic. (Amsterdam).* 107, 392–398. doi:10.1016/j.scienta.2005.11.001.

Staddon, P. L., Ramsey, C. B., Ostle, N., Ineson, P., and Fitter, A. H. (2003). Rapid turnover of hyphae of mycorrhizal fungi determined by AMS microanalysis of 14C. *Science* 300, 1138–40. doi:10.1126/science.1084269.

Stumpe, M., Carsjens, J.-G. G., Stenzel, I., Göbel, C., Lang, I., Pawlowski, K., et al. (2005).

Lipid metabolism in arbuscular mycorrhizal roots of Medicago truncatula. *Phytochemistry* 66, 781–791. doi:10.1016/j.phytochem.2005.01.020.

Tarafdar, J. C., and Marschner, H. (1994a). Efficiency of VAM hyphae in utilisation of organic phosphorus by wheat plants. *Soil Sci. Plant Nutr.* 40, 593–600. doi:10.1080/00380768.1994.10414298.

Tarafdar, J. C., and Marschner, H. (1994b). Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. *Soil Biol. Biochem.* 26, 387–395. doi:10.1016/0038-0717(94)90288-7.

Tawaraya, K., Naito, M., and Wagatsuma, T. (2006). Solubilization of insoluble inorganic phosphate by hyphal exudates of arbuscular mycorrhizal fungi. *J. Plant Nutr.* 29, 657–665. doi:10.1080/01904160600564428.

Tisserant, E., Kohler, A., Dozolme-Seddas, P., Balestrini, R., Benabdellah, K., Colard, A., et al. (2012). The transcriptome of the arbuscular mycorrhizal fungus Glomus intraradices (DAOM 197198) reveals functional tradeoffs in an obligate symbiont. *New Phytol.* 193, 755–769. doi:10.1111/j.1469-8137.2011.03948.x.

Torres de los Santos, R., Molinero Rosales, N., Ocampo, J. A., and Garc??a-Garrido, J. M. (2016). Ethylene Alleviates the Suppressive Effect of Phosphate on Arbuscular Mycorrhiza Formation. *J. Plant Growth Regul.* 35, 611–617. doi:10.1007/s00344-015-9570-1.

Turner, B. L. (2008). Resource partitioning for soil phosphorus: A hypothesis. *J. Ecol.* 96, 698–702. doi:10.1111/j.1365-2745.2008.01384.x.

van Diepen, L. T. A., Lilleskov, E. A., Pregitzer, K. S., and Miller, R. M. (2010). Simulated nitrogen deposition causes a decline of Intra- and Extraradical abundance of arbuscular mycorrhizal fungi and Changes in microbial community structure in northern hardwood forests. *Ecosystems* 13, 683–695. doi:10.1007/s10021-010-9347-0.

Vermue, E., Elbers, J., and Hoosbeek, M. (2008). A Comparative Field Study of Four Soil Respiration Systems. Available at: http://edepot.wur.nl/120659 [Accessed October 25, 2018].

Vestberg, M., Palojarvi, A., Pitkanen, T., Kaipainen, S., Puolakka, E., and Keskitalo, M. (2012). Neutral lipid fatty acid analysis is a sensitive marker for quantitative estimation of arbuscular mycorrhizal fungi in agricultural soil with crops of different mycrotrophy. *Agric. Food Sci.* 21, 12–27.

Vierheilig, H., Alt-Hug, M., Engel-Streitwolf, R., Mäder, P., and Wiemken, A. (1998a). Studies on the attractional effect of root exudates on hyphal growth of an arbuscular mycorrhizal fungus in a soil compartment-membrane system. *Plant Soil* 203, 137–144. doi:10.1023/A:1004329919005.

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

Watkins, N. K., Fitter, A. H., Graves, J. D., and Robinson, D. (1996). Carbon transfer between C3 and C4 plants linked by a common mycorrhizal network, quantified using stable carbon isotopes. *Soil Biol. Biochem.* 28, 471–477. doi:http://dx.doi.org/10.1016/0038-0717(95)00189-1.

Wewer, V., Brands, M., and Dörmann, P. (2014). Fatty acid synthesis and lipid metabolism in the obligate biotrophic fungus Rhizophagus irregularis during mycorrhization of Lotus japonicus. *Plant J.* 79, 398–412. doi:10.1111/tpj.12566.

Willis, A., Rodrigues, B. F., and Harris, P. J. C. (2013). The Ecology of Arbuscular Mycorrhizal Fungi. *CRC. Crit. Rev. Plant Sci.* 32, 1–20. doi:10.1080/07352689.2012.683375.

Yang, X., Post, W. M., Thornton, P. E., and Jain, A. (2013). The distribution of soil phosphorus for global biogeochemical modeling. *Biogeosciences* 10, 2525–2537. doi:10.5194/bg-10-2525-2013.

Zhang, L., Xu, M., Liu, Y., Zhang, F., Hodge, A., and Feng, G. (2016). Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. *New Phytol.* 210, 1022–1032. doi:10.1111/nph.13838.

Zhu, Y. G., and Miller, R. M. (2003). Carbon cycling by arbuscular mycorrhizal fungi in soil-plant systems. *Trends Plant Sci.* 8, 407–409. doi:10.1016/S1360-1385(03)00184-5.

Table 1. Description of the treatments tested by the time course experiment.

Treatment	Treatment Code	Fungal compartment (Total volume: 55 ml)	P content (mg)
Mycorrhizal plant without P	M+	Acid washed quartz sand (Awqs) (60 g) + milliQ water (16 ml)	0
Non mycorrhizal plant without P	M-	Awqs (60 g) + MilliQ water (16 ml)	
Mycorrhizal plant with goethite	GOE	Goethite (24.3 g) + milliQ water (28 ml)	
Mycorrhizal plant with orthophosphate bound to goethite (1.24 mg P/g)	GOE-OP	GOE-OP (24.3 g) + milliQ water (28 ml)	30
Mycorrhizal plant with phytic acid bound to goethite (1.79 mg P/g)	GOE-PA	GOE-PA (16.7 g) + Goethite (7.6 g) + milliQ water (28 ml)	
Mycorrhizal plant with orthophosphate (KH_2PO_4)	OP	Awqs (60 g) + MilliQ water (16 ml P solution)	
Mycorrhizal plant with phytic acid ($C_6H_{18}O_{24}P_6$)	PA	Awqs (60 g) + milliQ water (16 ml P solution)	

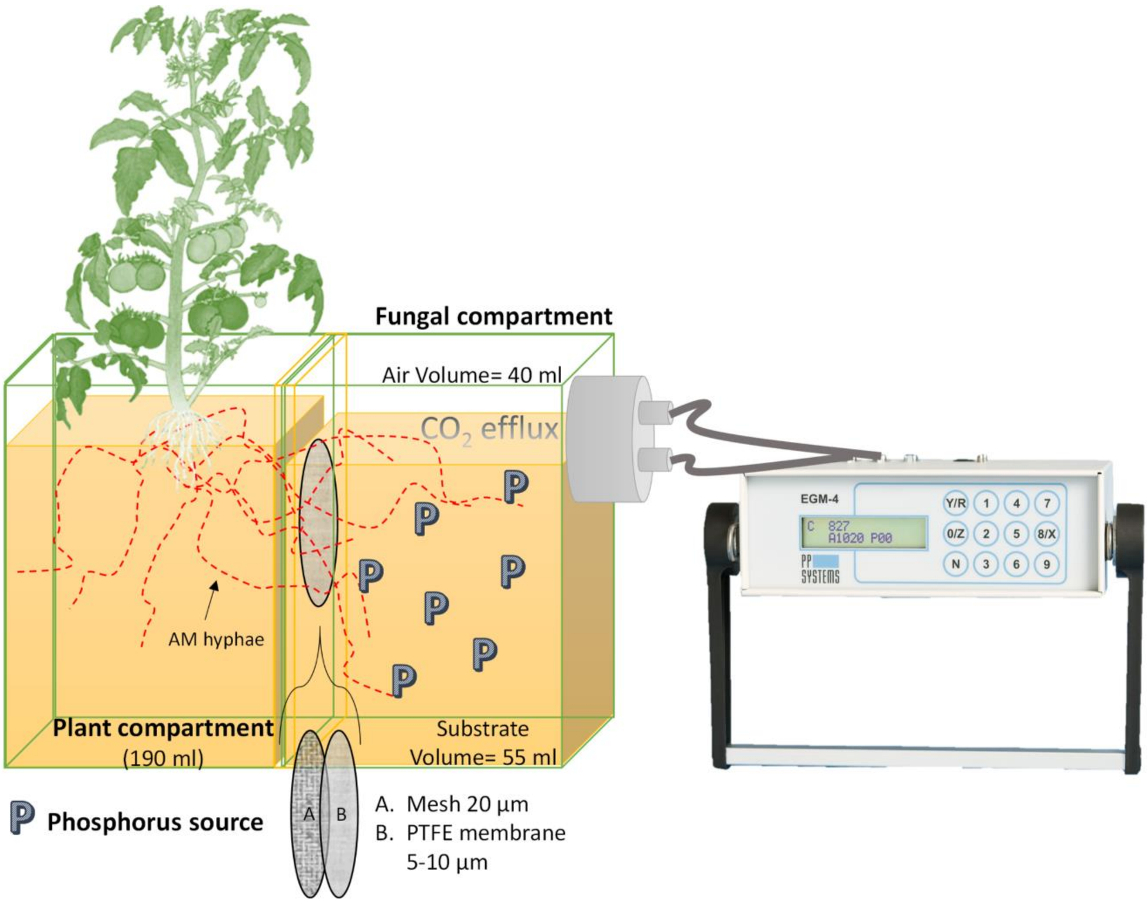


Figure 1. Mesocosm design. The mesocosms is made of a plant compartment (PC) linked to a fungal compartment (FC). Only the AMF is able to access the P sources located in the FC. In between both compartments, two membranes prevent roots from entering the FC, a 20 µm polyamide mesh and a polytetrafluoroethylene (PTFE) membrane with a pore size of 5–10 µm. The PTFE membrane does not allow mass flow and diffusion of ions to the PC. The FC is connected to the CO_2 measuring device (EGM-4) allowing its assembly every time a measurement is made.

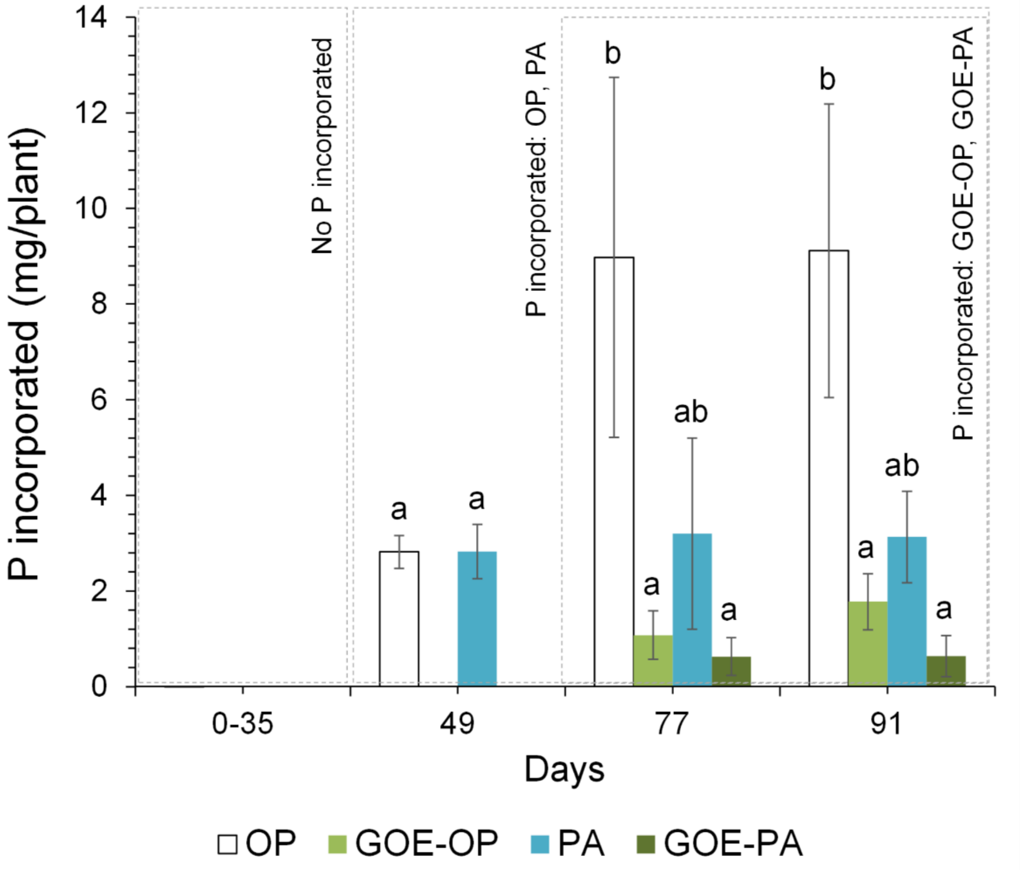


Figure 2. Total P (mg) incorporated by the AM tomato plant from the different P sources over 91 days. Each bar indicates the mean of three biological replicates and error bars are standard error. Within each P source and day, treatments with significant differences are labelled with different letters ($p < 0.05$) as result of a one-way ANOVA.

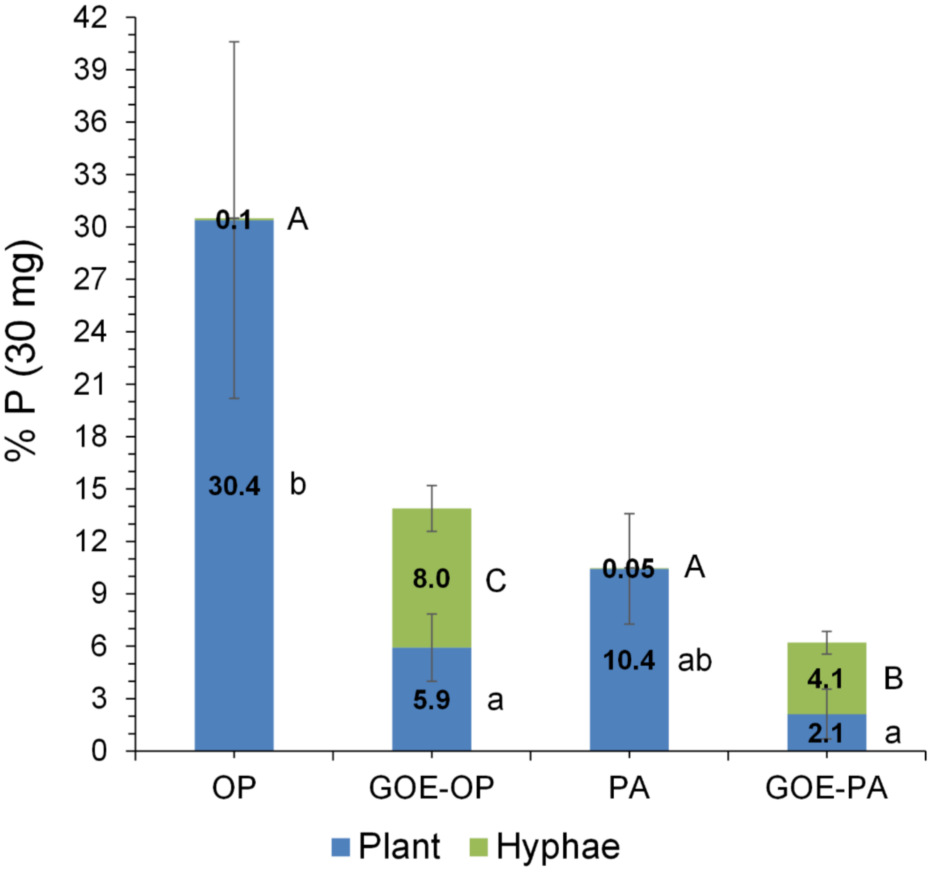


Figure 3. P stocks compartmentalized into plant tissues and external hyphae at day 91 (percentage of the total 30 mg P offered at the fungal compartment). Bars indicate the mean of three biological replicates and error bars are standard error. Within each P source and plant/hyphae, treatments with significant differences (P<0.05) are labelled with different lowercase letters for P% Plant stock and capital letters for P% hyphae stock lowercase as result of a one-way ANOVA.

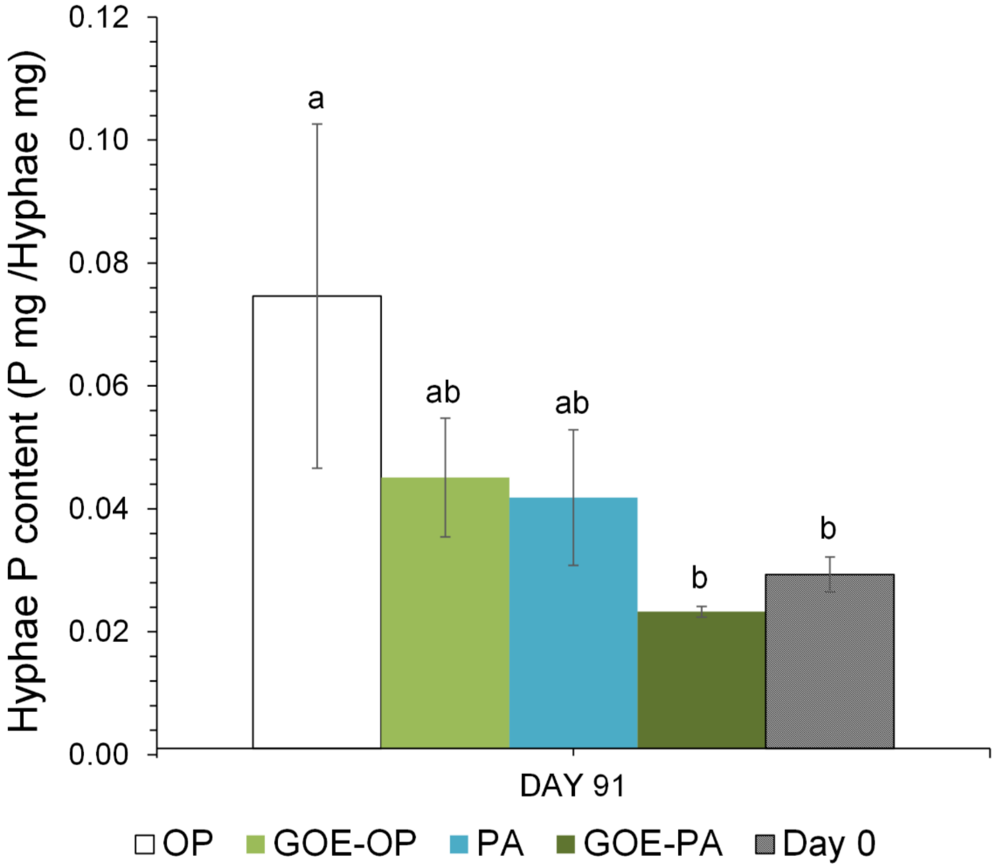
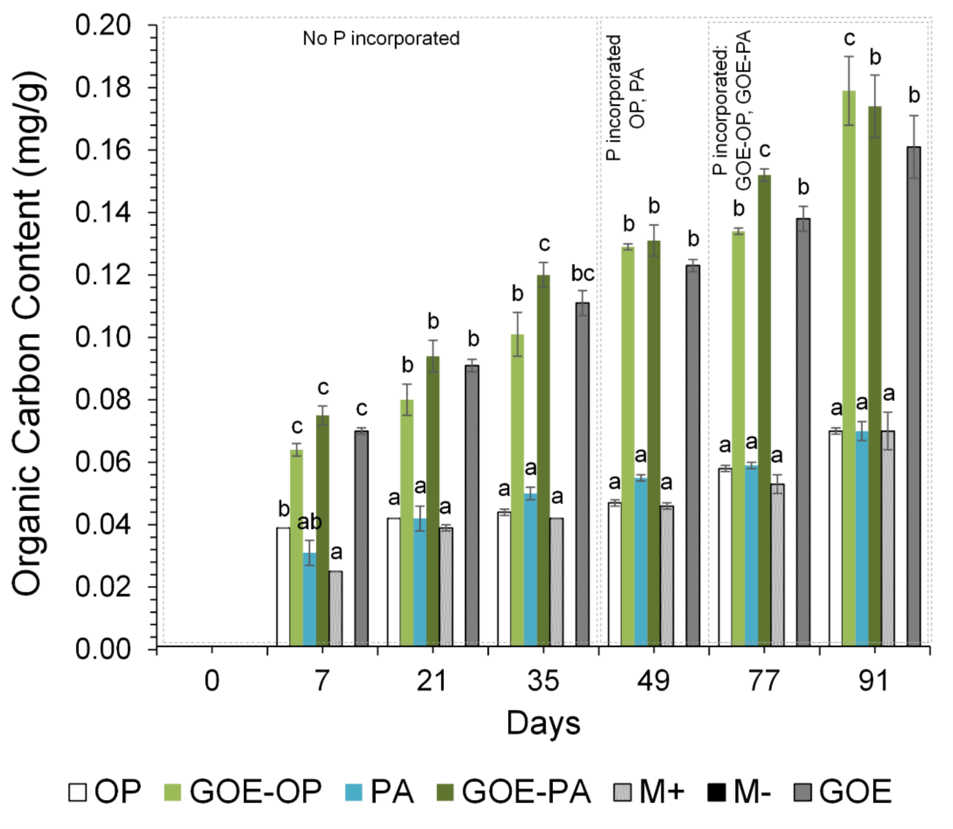


Figure 4. Total P content in the hyphae (P mg/Hyphae mg) grown at the fungal compartment for the different P sources on day 91, when the maximum P mobilisation was achieved in the four treatments. Each bar indicates the mean of three biological replicates and error bars are standard error. Within each P source, treatments with significant differences are labelled with different letters ($P<0.05$) as result of a one-way ANOVA.

A


0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
Organic Carbon Content (mg/g)
No P incorporated
P incorporated OP, PA
P incorporated: GOE-OP, GOE-PA
0
7
21
35
49
77
91
Days
□ OP
GOE-OP
PA
GOE-PA
M+
M-
GOE

B

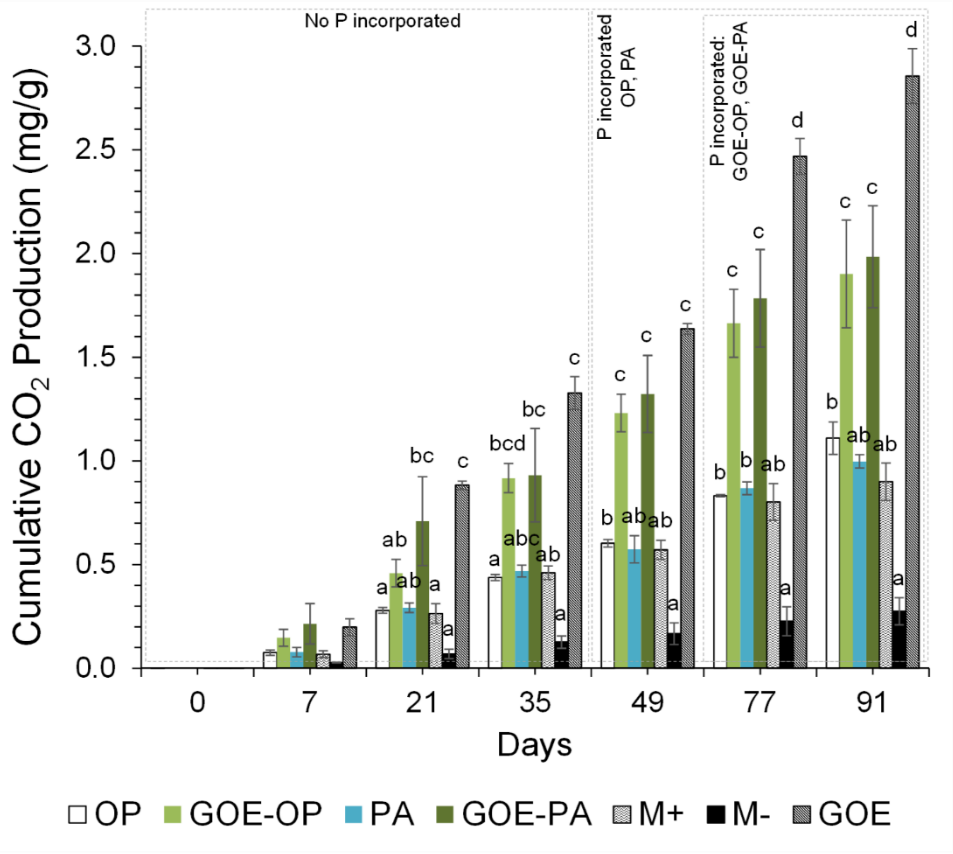

3.0
2.5
2.0
1.5
1.0
0.5
0.0
Cumulative CO_2 Production (mg/g)
No P incorporated
P incorporated OP, PA
P incorporated: GOE-OP, GOE-PA
0
7
21
35
49
77
91
Days
□ OP
GOE-OP
PA
GOE-PA
M+
M-
GOE

Figure 5. (A) Organic carbon content (mg/g fungal compartment). Each bar indicates the mean of three biological replicates and error bars are standard error. (B) Cumulative CO_2 production (mg/g fungal compartment). The bars show the mean of three biological replicates and error bars are standard error. Within each P source and day, treatments with significant differences are labelled with different letters ($P<0.05$) as result of a one-way ANOVA.

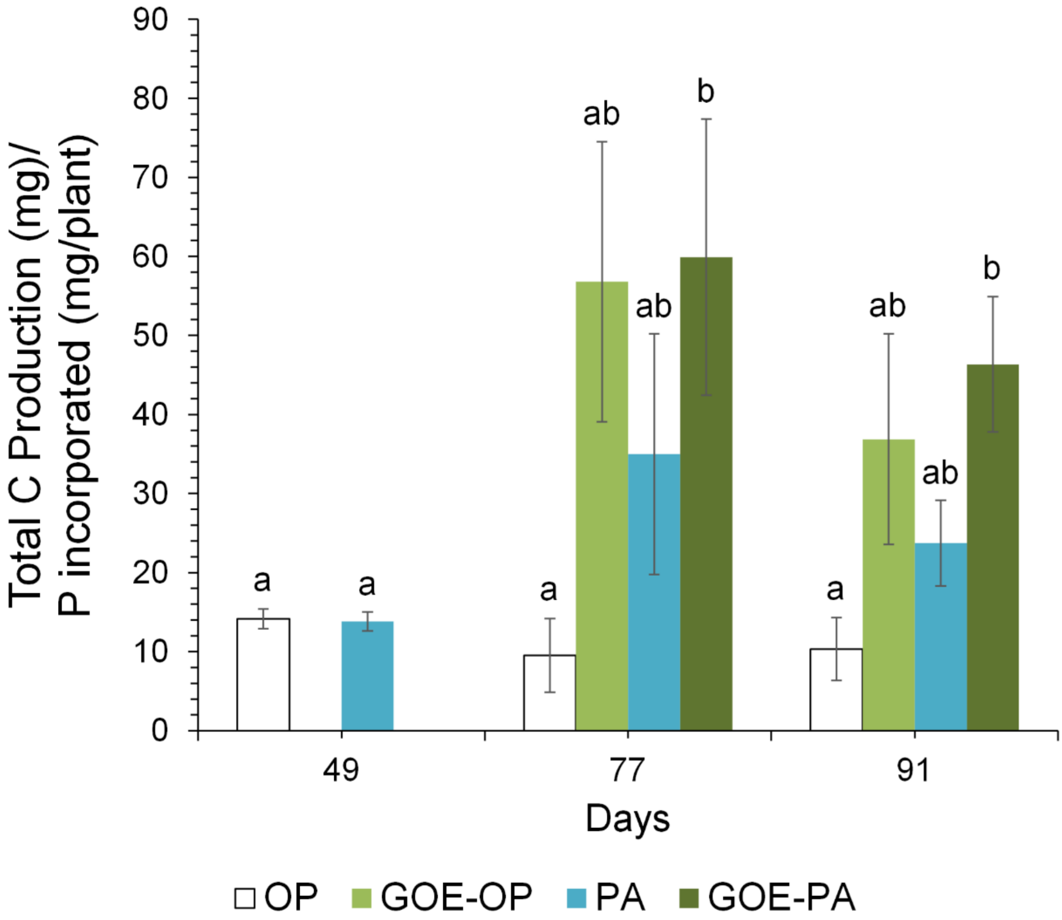
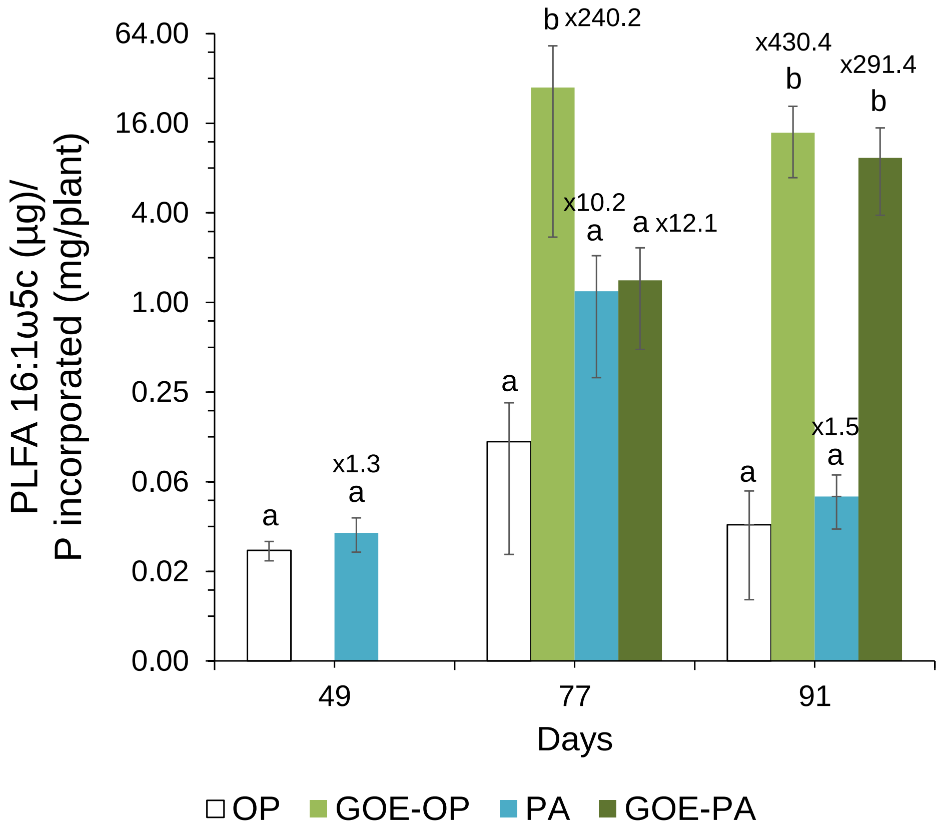
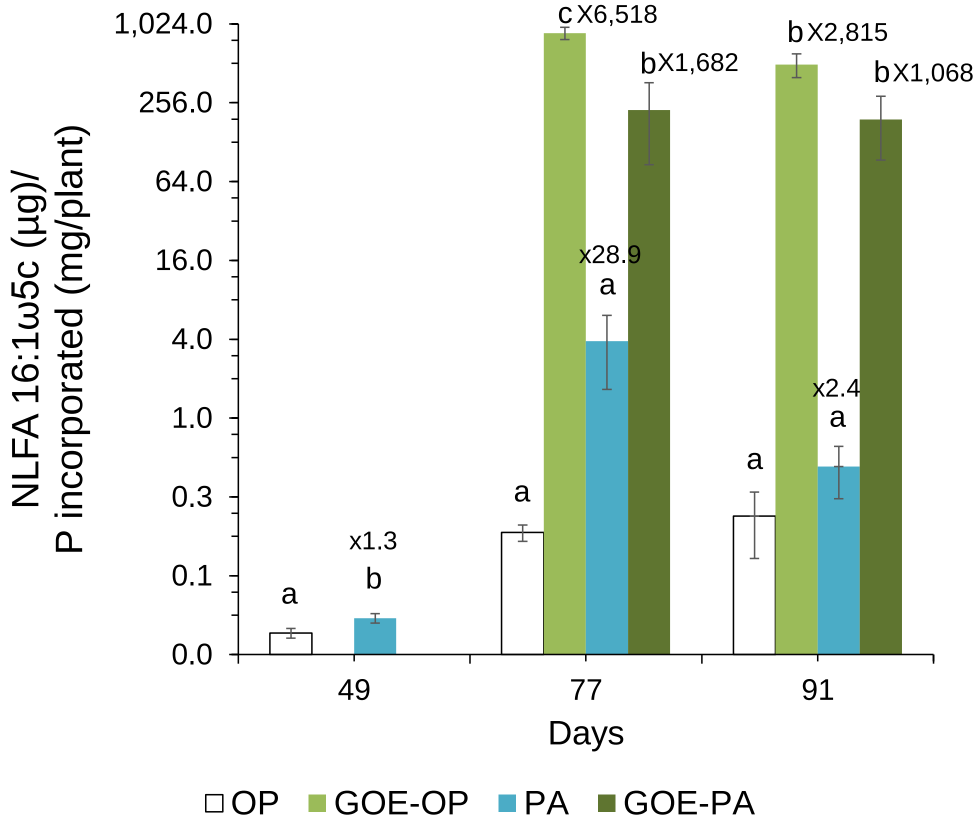


Figure 6. Mean values and standard errors (n=3) of the Total C/P ratio. Within each P source and day, treatments with significant differences are labelled with different letters ($P<0.05$) as result of a one-way ANOVA.

A


PLFA 16:1ω5c (µg)/
P incorporated (mg/plant)
64.00
16.00
4.00
1.00
0.25
0.06
0.02
0.00
a
x1.3
a
a
b x240.2
x10.2
a
a x12.1
x430.4
b
x291.4
b
a
x1.5
a
49
77
91
Days
☐ OP
GOE-OP
PA
GOE-PA

B


NLFA 16:1ω5c (µg)/
P incorporated (mg/plant)
1,024.0
256.0
64.0
16.0
4.0
1.0
0.3
0.1
0.0
a
x1.3
b
a
c X6,518
x28.9
a
b X1,682
b X2,815
a
x2.4
a
b X1,068
49
77
91
Days
☐ OP
GOE-OP
PA
GOE-PA

C

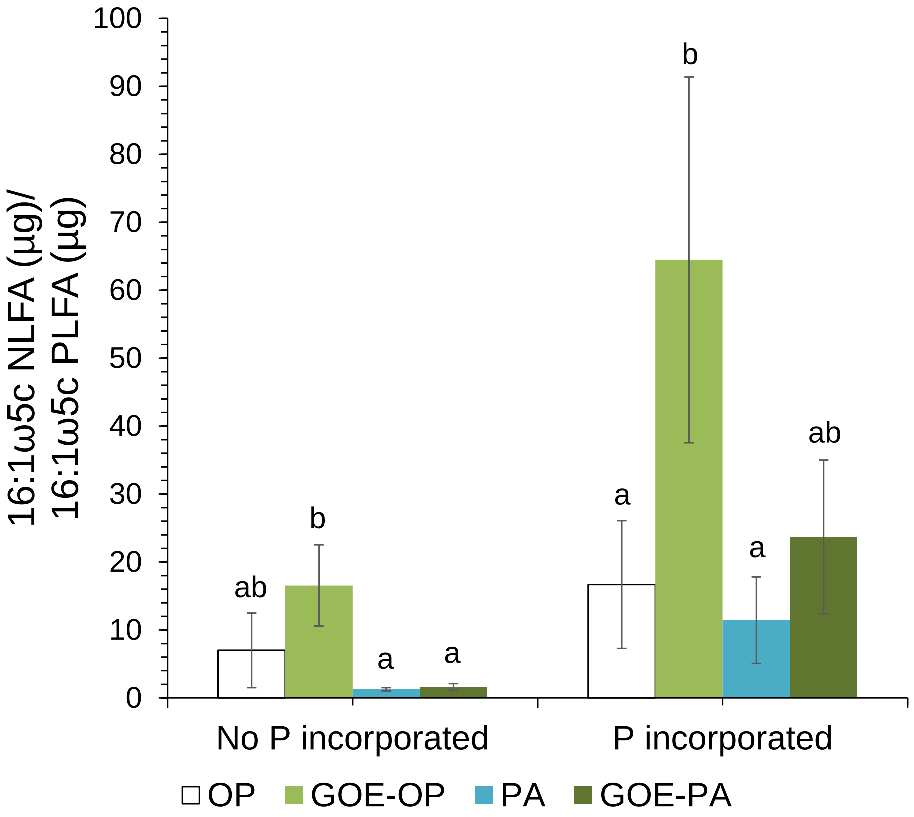


Figure 7. Ratios of PLFA 16:1ω5c to P incorporated within the tomato plant (A) and ratio of NLFA16:1ω5c to P incorporated within the tomato plant (B) from the different P sources along the time course experiment. Shown are mean values and standard errors (n=3). Within each P source and day, treatments with significant differences are labelled with different letters (P<0.05) as result of a one-way ANOVA. The numbers on top of each bar represent a multiplication factor compared to the P source with the lower ratio value. In all cases, the P source with the lowest ratios was OP. (C) Ratio of NLFA 16:1ω5c to PLFA 16:1ω5c. Shown are the mean values and standard errors of the NLFA 16:1ω5c/PLFA 16:1ω5c ratio for the sampling points belonging to the periods where no P incorporation was detected in the AM plant tissue as well as for those where we detected P in the AM plant. Within each P source, treatments with significant differences are labelled with different letters (P<0.05) as result of a one-way ANOVA.

Supplementary Material Study II

Carbon investment required for the mobilisation of inorganic and organic phosphorus bound to goethite by an arbuscular mycorrhiza (*Solanum lycopersic*um x *Rhizophagus irregularis*)

Alberto Andrino, Jens Boy, Robert Mikutta, Leopold Sauheitl, and Georg Guggenberger

Supplementary Tables and Figures

Supplementary Table 1. P-values for Figure 2. Total P (mg) incorporated by the AM tomato plant from the different P sources. Contrasts with significant differences are labelled with bold letters (P<0.05).

Contrast	Day 49	Day 77	Day 91
GOE-PA *vs* GOE-OP	1.000	0.892	0.966
OP *vs* GOE-OP	**0.001**	**0.032**	0.053
OP *vs* GOE-PA	**0.001**	**0.026**	**0.028**
PA *vs* GOE-OP	**0.001**	0.505	0.934
PA *vs* GOE-PA	**0.001**	0.427	0.732
PA *vs* OP	1.000	0.095	0.121

Supplementary Table 2. P-values for Figure 3.P stocks compartmentalized into plant tissues and external hyphae at day 91 (percentage of the total 30 mg P offered at the fungal compartment). Contrasts with significant differences are labelled with bold letters (P<0.05).

Contrast	p-value
GOE-OP *vs* GOE-PA	0.302
GOE-OP *vs* Day 0	0.449
GOE-OP *vs* PA	0.873
Day 0 *vs* GOE-PA	0.769
OP *vs* GOE-OP	0.172
OP *vs* GOE-PA	**0.028**
OP *vs* Day 0	**0.048**
OP *vs* PA	0.133
PA *vs* GOE-PA	0.377
PA *vs* Day 0	0.547

Supplementary Table 3. P-values for Figure 4. Total P content in the hyphae (P mg/Hyphae mg) grown at the fungal compartment for the different P sources on day 91, when the maximum P mobilisation was achieved in the four treatments. Contrasts with significant differences are labelled with bold letters (P<0.05).

Contrast	p-value
GOE-OP *vs* PA	**0.001**
GOE-OP *vs* OP	**0.001**
GOE-OP *vs* GOE-PA	**0.023**
GOE-PA *vs* PA	**0.019**
GOE-PA *vs* OP	**0.020**
OP *vs* PA	1.000

Supplementary Table 4. P-values for Figure 5. (A) Organic carbon content (mg/g fungal compartment). Each bar indicates the mean of three biological replicates and error bars are standard error. (B) Cumulative CO_2 production (mg/g fungal compartment). Contrasts with significant differences are labelled with bold letters (P<0.05).

A

Contrast	Day 7	Day 21	Day 35	Day 49	Day 77	Day 91
GOE *vs* GOE-OP	0.582	0.157	0.422	0.456	0.894	0.615
GOE *vs* M-	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE *vs* M+	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE *vs* OP	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE *vs* PA	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE-OP *vs* M-	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE-OP *vs* M+	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE-OP *vs* OP	**0.000**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE-OP *vs* PA	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE-PA *vs* GOE	0.795	0.996	0.597	0.183	**0.008**	0.866
GOE-PA *vs* GOE-OP	0.076	0.056	**0.024**	0.994	**0.001**	0.999
GOE-PA *vs* M-	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE-PA *vs* M+	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE-PA *vs* OP	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE-PA *vs* PA	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
M+ *vs* M-	**0.000**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.000**
OP *vs* M-	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.000**
OP *vs* M+	**0.015**	0.991	1.000	0.999	0.690	1.000
PA *vs* M-	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**0.000**
PA *vs* M+	0.615	0.985	0.718	0.094	0.612	1.000
PA *vs* OP	0.279	1.000	0.902	0.199	1.000	1.000

B

Contrast	**Day 7**	**Day 21**	**Day 35**	**Day 49**	**Day 77**	**Day 91**
GOE *vs* GOE-OP	0.980	**0.050**	0.101	0.061	**0.005**	**0.008**
GOE *vs* GOE-PA	1.000	0.797	0.120	0.214	**0.018**	**0.017**
GOE *vs* M-	0.161	**0.001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE *vs* M+	0.410	**0.003**	**0.001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE *vs* OP	0.474	**0.004**	**0.001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE *vs* PA	0.499	**0.004**	**0.001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE-OP *vs* M-	0.502	0.081	**0.001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE-OP *vs* M+	0.853	0.703	0.056	**0.001**	**0.003**	**0.006**
GOE-OP *vs* OP	0.900	0.769	**0.041**	**0.002**	**0.004**	**0.033**
GOE-OP *vs* PA	0.916	0.822	0.062	**0.002**	**0.006**	**0.013**
GOE-PA *vs* GOE-OP	0.927	0.442	1.000	0.986	0.991	1.000
GOE-PA *vs* M-	0.105	**0.002**	**0.001**	**< 0.0001**	**< 0.0001**	**< 0.0001**
GOE-PA *vs* M+	0.290	**0.036**	**0.047**	**0.001**	**0.001**	**0.003**
GOE-PA *vs* OP	0.342	**0.045**	**0.035**	**0.001**	**0.001**	**0.016**
GOE-PA *vs* PA	0.363	0.055	0.052	**0.001**	**0.002**	**0.006**
M+ *vs* M-	0.994	0.701	0.247	0.063	0.058	0.127
OP *vs* M-	0.985	0.631	1.000	**0.039**	**0.042**	**0.023**
OP *vs* M+	1.000	1.000	0.317	1.000	1.000	0.953
PA *vs* M-	0.980	0.570	0.228	0.060	**0.029**	0.058
PA *vs* M+	1.000	1.000	1.000	1.000	1.000	0.999
PA *vs* OP	1.000	1.000	1.000	1.000	1.000	0.998

Supplementary Table 5. P-values for Figure 6. Mean values and standard errors (n=3) of the Total C/P ratio. Contrasts with significant differences are labelled with bold letters (P<0.05).

Contrast	Day 49	Day 77	Day 91
GOE-OP *vs* GOE-PA	-	0.891	0.458
GOE-OP *vs* OP	**-**	**0.046**	0.061
GOE-OP *vs* PA	**-**	0.301	0.311
GOE-PA *vs* OP	**-**	0.055	**0.018**
GOE-PA *vs* PA	**-**	0.292	0.100
PA *vs* OP	0.864	0.234	0.302

Supplementary Table 6. P-values for Figure 7. Ratios of PLFA 16:1ω5c to P incorporated within the tomato plant (A) and ratio of NLFA16:1ω5c to P incorporated within the tomato plant (B) from the different P sources along the time course experiment. (C) Ratio of NLFA 16:1ω5c to PLFA 16:1ω5c. Contrasts with significant differences are labelled with bold letters (P<0.05).

A

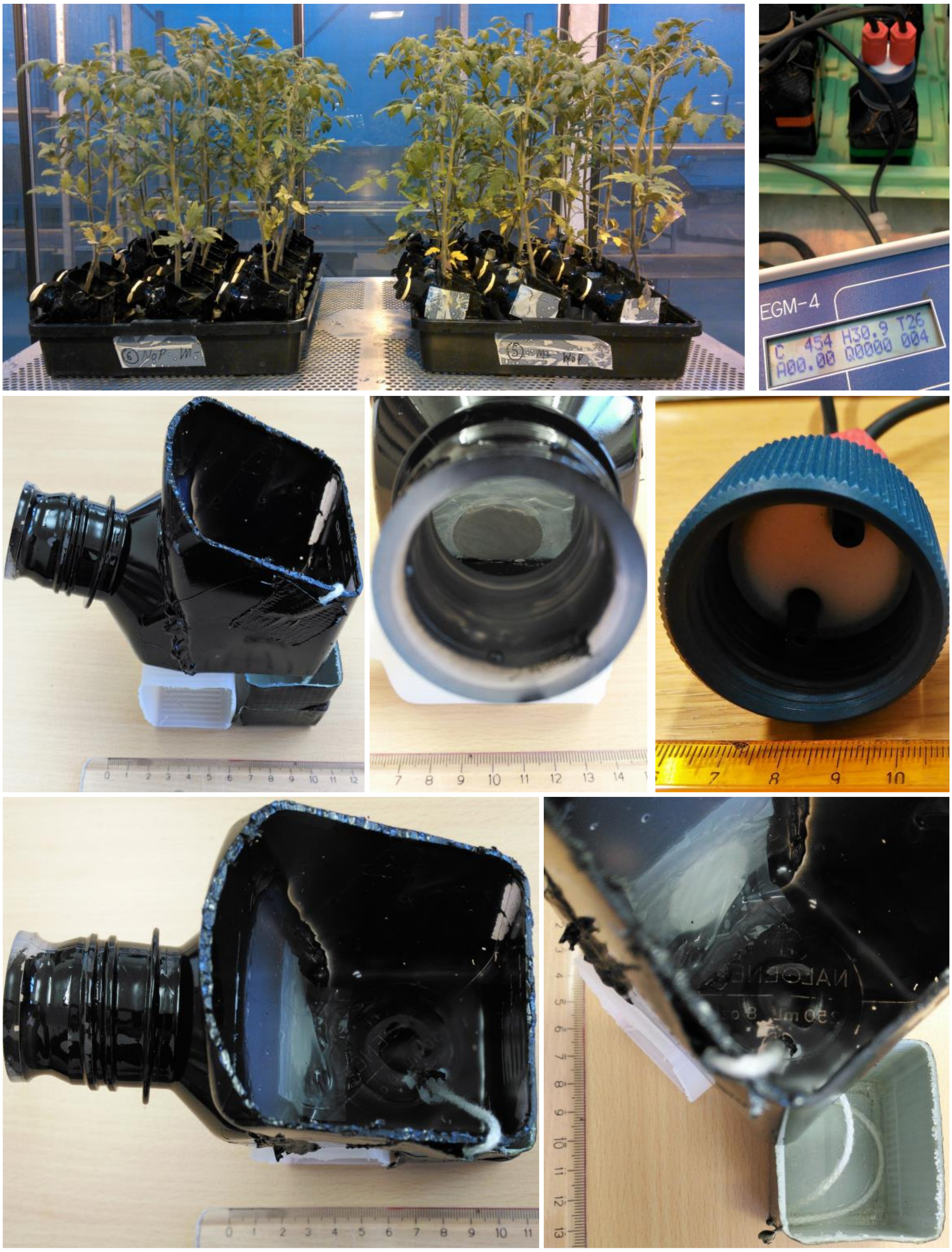
Contrast	Day 49	Day 77	Day 91
GOE-OP *vs* GOE-PA	1.000	**0.045**	0.736
GOE-OP *vs* OP	**0.022**	**0.026**	**0.035**
GOE-OP *vs* PA	**0.005**	**0.029**	**0.035**
GOE-PA *vs* OP	**0.022**	0.736	**0.021**
GOE-PA *vs* PA	**0.005**	0.788	**0.021**
PA *vs* OP	0.650	0.945	0.998

B

Contrast	Day 49	Day 77	Day 91
GOE-OP *vs* GOE-PA	1.000	**0.001**	0.062
GOE-OP *vs* OP	**< 0.0001**	**< 0.0001**	**0.001**
GOE-OP *vs* PA	**< 0.0001**	**< 0.0001**	**0.001**
GOE-PA *vs* OP	**< 0.0001**	**0.026**	**0.026**
GOE-PA *vs* PA	**< 0.0001**	**0.027**	**0.026**
PA *vs* OP	0.060	1.000	1.000

C

Contrast	No P incorporated	P incorporated
GOE-OP *vs* GOE-PA	**0.024**	0.099
GOE-OP *vs* OP	0.288	**0.036**
GOE-OP *vs* PA	**0.036**	**0.025**
GOE-PA *vs* OP	0.804	0.982
GOE-PA *vs* PA	1.000	0.922
OP *vs* PA	0.805	0.991



Supplementary Figure 1. Mesocosms experiment. From bottom to top, the lower pictures show different details of the mesocosm design, the wick irrigation system and a close-up view of the polyamide mesh. Middle pictures, the picture on the left shows a mesocosm with the irrigation compartment. The picture in the middle shows the threaded section of the fungal compartment and a close-up view of the polytetrafluoroethylene membrane. The image on the right shows the lid that connects to the EGM-4 and houses the CO_2 inlet and outlet tubes. The upper right image shows a measurement with the EGM-4 in operation and the one the left shows some mesocosms containing the tomato plants and the black nylon cloth with a 0.5 mm pore on the mouth of the fungal compartment

4. Study III

Mechanisms to mobilise phosphorus bound to an iron oxide by an arbuscular mycorrhiza (*Solanum lycopersicum x Rhizophagus irregularis*)

Alberto Andrino[1], Jens Boy[1], Robert Mikutta[2], Sarmite Kernchen[3] and Georg Guggenberger[1]

[1]Institute of Soil Science, Leibniz Universität Hannover, Herrenhäuser Straße 2, 30419 Hannover, Germany.
[2]Soil Science and Soil Protection, Martin Luther University Halle-Wittenberg, von-Seckendorff-Platz 3, 06210 Halle (Saale), Germany.
[3]Bayreuth Center of Ecology and Environmental Research (BayCEER), Bayreuth University, Dr. Hans Frisch Straße B3, 95448 Bayreuth, Germany.

Author contribution

JB, GG, AA, and RM designed the experiment. AA prepared the plant and fungal material. AA conducted the experiment and analysed the data. SK analysed the LMWOA on the fungal compartment samples. JB, GG, and RM supervised the research. AA wrote the paper with contributions from JB, GG, RM and SK.

Prepared for submission

Abstract

Arbuscular mycorrhiza fungi (AMF) establish symbiotic associations with most of the terrestrial plants, thus enhancing phosphorus (P) availability. While studies on P supply by inorganic P abound, there is a lack of knowledge on the ability of AMF to mobilise P sources bound to secondary minerals. The aim of the study was to elucidate the mechanisms involved in the mobilisation of orthophosphate (OP) or phytic acid (PA) from sorption sites of goethite, a common iron (Fe) oxide in soils, relative to respective free P sources. Mesocosms experiments with *Solanum lycopersicum* L. plants mycorrhized with the AMF *Rhizophagus irregularis*, were run in a two-compartment design, with a fungal compartment where P sources were exclusively accessible by the AMF. In the plant compartment N and P contents, photosynthesis, and mycorrhization rates were determined while at the fungal compartment low-molecular weight organic acids (LMWOA) and fatty acid microbial biomarkers were monitored. All P sources were incorporated into the AM plants, but amounts and uptake rates differed. AM plants with access to a P source preferentially stored it in the shoot influencing positively the photosynthetic function, by showing an improved photosynthetic P use efficiency. P incorporation in AM plants correlated with AMF biomarkers together with gram-positive bacteria for treatments containing PA and GOE-PA, but not for GOE-OP. We found bacterial or fungal LMWOA with two and three carboxylic groups in the treatments containing PA, GOE-PA, and GOE-OP before and during P incorporation, pointing to a ligand exchange mechanism to mobilise the P.

Keywords

LMWOA, photosynthesis, N:P ratio, organic phosphorus, inorganic phosphorus, arbuscular mycorrhiza, PLFA 16:1ω5c, NLFA 16:1ω5c, membrane fluidity, goethite.

Introduction

Phosphorus (P) is an essential plant macronutrient (Schachtman et al., 1998) and its deficiency limits plant growth in both natural systems and agriculture (Oberson et al., 2001). The high affinity and strong specific adsorption of inorganic and organic P forms to Fe oxides determines their desorption and, hence, availability to plants (He and Zhu, 1998). The dynamics of organic P forms in soils is much less understood compared to inorganic P forms such as orthophosphate (OP). Most studies on plant nutrition often consider only inorganic P to be biologically available but not the organic fraction, like the myo-inositol hexakisphosphate stereoisomer or phytic acid (PA) (Turner, 2008), despite of PA being the most common form of inositol phosphates in soils and representing >50% of the organic P in soils (Ognalaga et al., 1994). PA has a greater affinity than OP for Fe oxides, as the high anionic charge of PA facilitates the formation of strong electrostatic bonds (Nash et al., 2014).

Arbuscular mycorrhizal fungi (AMF) establishes a symbiotic association with most of the terrestrial plants, enhancing the availability of nutrients, especially P, by forming an extensive mycelium network that operates as functional extensions of the plant root system (Xu et al., 2007). In exchange for the acquired P, AMF receive carbon (C) from the host plant (Zhang et al., 2016), mostly in the form of carbohydrates and lipids. AMF lack genes for *de novo* lipid biosynthesis enzymes and can only elongate 16C lipid molecules, thus requiring the plant to produce them (Bravo et al., 2017; Jiang et al., 2017; Keymer et al., 2017; Luginbuehl et al., 2017), and contributing to the obligate biotrophy of the fungus (Nadal and Paszkowski, 2013; Wewer et al., 2014). The C supply to the AMF results in an enhanced plant photosynthetic activity by wider opening of the stomata, allowing for more CO_2 to diffuse into the leaf and increased sucrose hexose level in the roots (Boldt et al., 2011). This is needed as photosynthesis is regulated by C demand and the mycorrhizal roots represents a strong C sink (Rich et al., 2017). An increased C demand stimulates photosynthetic activity; the strength of the new C sink accelerates the utilization of triose phosphate for sucrose synthesis and the export towards the phloem, thus increasing plant P recycling rates when releasing P back to the chloroplast and activating the regeneration of Ribulose 1,5-bisphosphate in the Calvin Cycle. Therefore, more C is fixed per time and per unit of P, resulting in higher photosynthetic P use efficiency (Kaschuk et al., 2009; Tuomi et al., 2001).

There is a growing body of literature stating that after the plant photoassimilates reach the fungal symbiont, AMF act as a hub for translocating fresh plant-derived C to soil microbes. Then, the C input affects the diversity, structure and the strength of AMF-associated microbial communities (Agnolucci et al., 2015; Choudhary et al., 2017; Gui et al., 2017; Kaiser et al., 2015; Krishnamoorthy et al., 2016; Nuccio et al., 2013; Paterson et al., 2016; Toljander et al., 2007).

The surface of mycorrhizal hyphae and spores are a stable microhabitat for the establishment of bacterial biofilms in soil (Manchanda et al., 2017) and certain types of bacteria are known to attach specifically to AMF hyphae, indicating different ability to attach between different bacterial groups (Taktek et al., 2017). This specific attachment may be indicative for further colonisation and more complex types of interactions between bacteria and hyphae (Scheublin et al., 2010). The hyphosphere, the zone surrounding individual fungal hyphae, is a C-rich environment but deficient in available P for many microbes, which may stimulate their activity in mineralizing organic P forms (Jansa et al., 2013). P mobilisation may be a win-win strategy among AMF hyphosphere and its associated bacteria (Drigo and Donn, 2016; Wang et al., 2016; Zhang et al., 2014). A considerable body of literature has investigated the joint action of AMF with groups of mineralizing and solubilizing bacteria with the potential to improve P availability to the plant through synthetized phosphatases (E.g., acid phosphatase, phytase), lowering the pH of the soil, and/or ligand exchange reactions mediated by low-molecular-weight organic acids (LMWOA) (Dames, 2014; Giasson et al., 2005; Giri et al., 2005; Hoberg et al., 2005; Hwangbo et al., 2003; Igual et al., 2001; Mishra et al., 2016; Ordoñez et al., 2016; Zhang et al., 2016). The accompanying AMF microbiota can be functionally diverse. For example, Battini et al. (2016) isolated microbiota from *Rhizophagus intraradices* mycelium and found plant-growth promoting activities such as phytate mineralization, siderophore production, mineral P solubilisation, and indol acetic acid production in several representatives of gram-positive (E.g. *Streptomyces* spp., *Arthrobacter* spp., *Nocardiodes* spp., *Bacillus* spp.) and gram-negative bacterial groups (E.g. *Sinorhizobium* spp.). Individually, the AMF possess the ability to absorb OP directly from the soil solution (Smith et al., 2003) and mineralize organic P through the action of AMF acid phosphatases, which are able to act on phosphate esters (Joner et al., 2000; Koide and Kabir, 2000; Sato et al., 2015; Tarafdar et al., 2002; Tarafdar and Marschner, 1994a, 1994b; Tisserant et al., 2012). There is also evidence that AMF can desorb OP from ferrihydrite (Rakshit and Bhadoria, 2010; Virant-Klun and Gogala, 1995), but not goethite (α-FeO(OH)), one of the most abundant iron (oxy)hydroxides (Giles et al. 2012). The release of P bound to pedogenic oxides requires a chelation step by the action of LMWOA (Geelhoed et al., 1999), but production of LMWOA by AMF is still poorly documented (Bharadwaj et al., 2012; Giasson et al., 2005; Manaut et al., 2014).

In the previous Study 2 (Andrino et al., Submitted), we already showed that the different P sources were mobilised by *R. irregularis* and in the current study we aim at investigating the mechanisms involved in the mobilisation of organic and inorganic P sources bound to goethite by AMF. The PA, OP, and goethite (GOE) were selected due their common abundance in soils worldwide (Giles et al., 2012; Shen et al., 2011; Turner, 2008). Especially in (sub) tropical ecosystems, Fe oxides like goethite and hematite promote soil P saturation, and diminish P solubility and plant availability, respectively. To elucidate the mechanisms involved in P acquisition, both P sources (OP, PA) were tested individually and bound to goethite in a time course experiment using a two-compartment mesocosm, where only the AMF was able to access the different P sources placed into the fungal compartment. In the plant compartment, we determined plant biomass, plant P and nitrogen content, photosynthetic capacity, and mycorrhizal root status. In the fungal compartment, we measured low-molecular-weight organic acids (LMWOA) and the putative fatty acid biomarkers for monitoring the composition of the microbial communities grown in presence of the different P sources.

We hypothesize that (1) AM plants accessing the different P sources will mobilise P in different amounts and rates, translocating P into the plant tissues (2) P mobilisation will have an impact on the photosynthetic function, which will be enhanced in case AMF accessing a P source, in order to keep up a constant transfer of photosynthates to the AMF. We further speculate (3) that the AMF growth will always correlate positively with P incorporation into the plant tissues for all P sources, and that (4) the P mobilisation from the different sources has an impact on the profile of LMWOA produced at the fungal compartment, either from AMF or from associated bacteria. Here, we expect LMWOA with two and three carboxylic groups being more abundant in case of the less accessible mineral-associated P compounds.

Material and methods

Plant Mycorrhization

Solanum lycopersicum L. var. Moneymaker seeds (Volmary GmbH) were surface-sterilized (H_2O_2 5%, 10 minutes), soaked in distilled, and autoclaved water and pre-germinated on petri dishes (72 hours, 27°C). As AMF we selected *R. irregularis* DAOM 197198 due to its global distribution and well adaptation to intensive agricultural practices (Köhl et al., 2016). The inoculum consisted of 0.4 g of spores, hyphae and root fragments of *Sorghum bicolor* inoculated with *Rhizophagus irregularis* DAOM 197198 (Symplanta GmbH & Co. KG) grown in a trap plant culture following the methodology of Brundrett (1996). The combination of both organisms has been selected in several others research studies as a model of mutualistic association (Fernández et al., 2014; Herrera-Medina et al., 2008; Morcillo et al., 2016; Tienebo, 2016). Tomato pre-germinated seeds were planted in QP96 cells (HerkuPlast Kubern GmbH) together with the *R. irregularis* inoculum and 70 ml autoclaved and acid washed quartz sand (Sahara Spielsand ^{TM}). The quartz sand was used as a nutrient free culture substrate suitable for the colonization of AM fungi, where high-purity mycelium can develop (Johansen et al., 1996; Olsson and Johansen, 2000). *S. lycopersicum* mycorrhizal and non-mycorrhizal plants were grown at a greenhouse (16/8 light/dark, 24/20°C light/dark, 50–60% relative humidity, photon flux density of 175–230 µmol/m/s). *S. lycopersicum* seedlings were watered every day with 10 ml deionized water and on alternate days were fertilized with 5 ml low P (0.32 mM) modified Long Ashton nutrient solution pH 6.5 (Hewitt, 1966).

Time Course Experiment

The first harvest took place at the planting day into the mesocosm (day 0), to determine the initial P content of the plants (n=5), followed by harvest points at day 7, 21, 35, 49, 77, and 91. The mesocosms were made of two compartments, a plant compartment and a fungal compartment. In the latter, exclusively hyphae could enter and access the four P sources (Figure 1). A polyamide mesh (20 µm pore diameter) (Franz Eckert GmbH) separated mycorrhizal roots and mycelium (Fitter et al., 1998; Watkins et al., 1996), while the polytetrafluoroethylene membrane (5-10 µm pore diameter) (Pieper Filter GmbH) allowed the AMF hyphae to cross but avoided the diffusion of ions into the plant compartment (Mäder et al., 1993, 2000; Vierheilig et al., 1998a). The P sources were placed into the fungal compartment as described in Table 1. Four-weeks old mycorrhizal and control *S. lycopersicum* plants were planted into the plant compartment. Mesocosms, comprising three biological replicates per P source and harvest point, were placed in a climatic controlled greenhouse (24/20°C light/dark; photoperiod 16/8 h light/dark; 50–60% relative humidity; photon flux density of 175–230 $\mu mol/m^2/s$). They were watered two times a week with 10 ml MilliQ water. On alternate days, the pots were fertilized with 5 ml no-P Long Ashton nutrient solution. Once a week, the mesocosms were rotated to achieve homogeneous growth conditions for all mesocosms.

Phosphorus Sources

Four P sources were added into fungal compartments as described in Table 1. Orthophosphate was added as KH_2PO_4 (Sigma-Aldrich, Steinheim, Germany) whereas PA was added as sodium salt (Sigma-Aldrich, Steinheim, Germany). The adsorption complexes were prepared by equilibrating P compounds with goethite (Bayferrox 920 Z). The first step involved the equilibration of 50 g of goethite for 16 h in 250 ml MilliQ H_2O (pH 4). Second, 250 ml MilliQ H_2O containing either 17 g KH_2PO_4 or 0.72 g $C_6H_{18}O_{24}P_6$ were added to the goethite solution and equilibrated for 48 h on an overhead shaker. The goethite-P suspensions were centrifuged for 15 min (3000 g) and pellets were afterwards rinsed with MilliQ H_2O until the electric conductivity was <40 µS/cm. The resulting goethite-P associations were shock-frozen in liquid N_2, and freeze-dried. The loading of OP and PA onto the goethite were determined by hydrolysing 5 mg of the goethite-P associations in concentrated HNO_3 (n=3) and subsequent measurement of P contents by ICP-MS Agilent 7500C (Agilent Technologies, Santa Clara, CA, USA). The adsorption complexes contained 1.24 mg P/g in case of OP (GOE-OP) and 1.79 mg P/g for PA (GOE-PA), respectively.

Plant biomass, phosphorus and nitrogen contents

At each harvest point, shoots and roots were dried (70°C, 48 h), weighed, ball milled, and P contents were determined. An aliquot of shoot and root were incinerated at 480°C for 8 h, digested in 1 ml 30% HNO_3, filtered to <0.45-µm (PVDF filters), and the solutions analysed by ICP-MS Agilent 7500C (Agilent Technologies, Santa Clara, CA, USA). Total N content from the milled shoot material was measured by dry combustion using an Elementar vario MICRO cube C/N Analyser (Elementar GmbH, Hanau, Germany). Shoot and root P contents in percentage of total plant dry weight (%dw) were calculated for each time point and treatment. Total P incorporated into the plant tissues was calculated by subtracting the total P content (mg) in a subsample (n=5) of the initial transplanted AM plants at day 0 and the total P content (mg) at each harvest point. The shoot N:P ratio, an indicator for P deficiency in the shoot tissues (Hayes et al., 2014; Koerselman and Meuleman, 1996; Ros et al., 2018), was calculated for plants accessing the different P sources and controls (Eq. 1). Shoot N:P ratios are useful to investigate shifts from N to P limitation because they are easily determined and compared across studies, N limitation for terrestrial plants occurs at values below 10, while P limitation usually occurs above 20 and may cause the inhibition of photosynthesis (Güsewell, 2004).

$$\text{N:P ratio} = \frac{\text{Total N in the shoot (mg)}}{\text{Total P in the shoot (mg)}} \tag{1}$$

Photosynthetic Capacity

At each harvest point, the photosynthetic capacity was measured on recently fully expanded third or fourth leaf from the top, in order to check the impact of the different P sources on the host carbohydrate metabolism, as P deficiency causes a rapid decrease in photosynthetic capacity (Maathuis, 2009). At each time point, CO_2 assimilation rate was measured with the LI-6400 (LiCor, Lincoln, NE, USA). Values were recorded at 22°C in the leaf cuvette, at approx. 50% relative humidity, airflow rate of 400 µmol/s, an external CO_2 concentration of 360 ppm, and the CO_2 mixer was set at 400 ppm. Irradiance was provided by a led source set to a photon flux density of 1000 µmol/m^2/s.

Visual Quantification of Mycorrhizal Root Status

Before planting the seedlings into the mesocosms and at each harvest point, a root subsample was digested with 10% KOH (35 min, 95°C) and stained using the ink and vinegar staining technique for AMF (Vierheilig et al., 1998b). Mycorrhization rates were determined using the methodology from McGonigle et al. (1990). Arbuscules, vesicles, and intraradical mycelium were expressed as percentage.

Low-Molecular-Weight Organic Acids

Concentration and composition of LMWOA in the fungal compartment containing the different P sources were analysed in order to determine their role in P acquisition from the different sources. LMWOA are involved in P solubilisation also known as ‘organic acid theory’ (Owen et al., 2015). There are two mechanisms of P acquisition involved, on the one hand, by lowering pH by proton extrusion. On the other hand, by the release of LMWOA that exchange for P on soil adsorption sites. The presence of LMWOA in soil can displace adsorbed P through ligand exchange reactions into soil solution and, by this, increase P uptake rates. The mechanism to mobilise oxide-bound P requires a chelation step, which involves the formation of two or more coordinate bonds between a ligand molecule and a metal ion, thereby creating a ring structure complex (Geelhoed et al., 1999; Richardson, 2001). The ability of different carboxylic anions to desorb P generally decreases with a decrease in the stability constants of Fe (III) acid complexes. Tricarboxylic acids such citrate have a higher efficiency than dicarboxylic or monocarboxylic acids (Deubel and Merbach, 2005; Field and Marschener, 1998; Jones, 1998). LMWOA were determined by the method of Tani et al. (1999). In brief, the crude sample was extracted from a moist sample of 5 g of each fungal compartment with NH_4-phosphate buffer (0.1 M $NH_4H_2PO_4$-H_3PO_4, pH 2) at a sample (related to dry sample) to solution ratio of 1:4, by shaking for 30 min on a horizontal shaker. Then, the crude extract was separated from the sample by centrifuging at 10,000 g for 10 min, followed by further filtration through a 0.025 µm Millipore filter. Filtered extracts were analysed with an Agilent series 1100 liquid chromatograph (Agilent Technologies, Santa Clara, CA, USA) coupled to electrospray ionization (ESI) mass spectrometer (Agilent 6130 single quadrupole) to determine the different LMWOAs. The HPLC system was equipped with a degasser, quaternary pump, autosampler, and a column thermostat set at 40 °C. Separation of LMWOAs was achieved on a hydro-reversed-phase column Phenomenex Synergi 4 u Hydro-RP 80A, 250 × 3 mm^2 combined with a guard column (4 × 2.0 mm^2) of the same packaging material from Phenomenex with 4 mM formic acid (solvent A) and MeOH (solvent B) as mobile phase. The LC system was routinely operated in a gradient mode and every sample was run with two chromatographic methods, which were found most sensitive for a number of selected LMWOAs. Thus, acetic, propionic, butyric, *iso*-butyric, and oxalic acid were analysed with a post column addition of 1 M aq. ammonia (method I) and succinic, citric, lactic, maleic, D-gluconic, malic, glycolic, tartaric, malonic, and 2-keto-D-gluconic acid were analysed with the post column addition of methanol (method II). Method I was performed using a gradient at a mobile phase flow rate of 0.2 ml min^{-1} as follow: the gradient started with 16% B for 10 min, then in a linear gradient reached 80% B at 35 min and returned to the initial conditions over 8 minutes. Chromatographic method II was run with the mobile phase flowing at a rate of 0.4 mL min^{-1} with LC conditions starting with 4% B for 10 min, followed by a gradient up to 80% over 23 minutes and returning to 4% in a post time over 7 minutes. Post column addition of 1 M aq. ammonia or methanol was performed by a micro pump (MPLC, model G, Brownlee Labs, San Francisco, USA) set at 10% flow rate of the eluent, i.e., 20 µL min^{-1} or 40 µL min^{-1}, respectively. For each chromatography experiment, 900 mL of sample was mixed with 100 mL of internal standard solution (2-furoic acid, 1 mM) and 50 µL of the resulting solution was injected. The single quadrupole MS was operated in the negative ionization mode. Monitoring of ions was carried out in the molecular mass range 50–500 Da to examine the fragmentation of organic acids. Quantification and identification of the separated analytes was done in the selected ion monitoring (SIM) mode primarily by matching the retention times of standards. ESI parameters were sequentially optimized via flow injection analysis using 50 µM standard solution of each LMWOA. The following parameters yielded highest signal intensities and were applied for all chromatographic runs: nebulizer gas (N_2) 35 psi; drying gas (N_2) flow rate 10 L min^{-1}; capillary voltage 2500 V; gas temperature 350 ^{o}C. The fragmentor voltage was set at 70 V. External standards within the range 0.01–1000 µM were used for calibration, and the data were processed using Agilent's Chemstation software package. Methanol (HPLC grade), formic acid (≥98%), and water (HPLC grade) were acquired from Carl Roth GmbH & Co (Karlsruhe, Germany). Acetic acid (≥99%), maleic acid (99%), D-gluconic acid sodium salt (98%), oxalic acid (≥99%), ammonium tartrate dibasic (≥99.5), 2-furoic acid (98%), and malonic acid disodium salt (≥98%) were acquired from Sigma-Aldrich (Steinheim, Germany). Citric acid monohydrate (extra pure), DL-malic acid (>99.5%), and glycolic acid (>99%) were obtained from Merck (Darmstadt, Germany). Propionic acid (99%), butyric acid (99%), *iso*-butyric acid (99%), lactic acid (98%), succinic acid (99%), and 2-keto-D-gluconic acid hemicalcium salt (99%) were acquired from Alfa Aesar GmbH & Co KG (Karlsruhe, Germany). Stock solutions of each standard (50 mM) were prepared in HPLC grade water and stored at -20 ^{o}C until usage. For each P source, the mean concentration of each LMWOA was calculated for the periods where no P incorporation was detected in the AM plant tissue as well as for those where we detected P uptake in the AM plant.

Fatty Acid Analysis in the Fungal Compartment

Analysis of the phospholipid fatty acid (PLFA) and the neutral lipid fatty acid (NLFA) were used to assess the microbial community structure (Zelles, 1999) in the fungal compartments containing the different P sources. Seventeen different fatty acids were analysed for their microbial group designation (Table 2). Lipids were extracted from 8 g samples of the fungal compartments containing goethite or 16 g for the ones with quartz and fractionated into PLFA and NLFA by solid phase extraction with activated silica gel (Sigma Aldrich, pore size 60 Å, 70–230 mesh). The PLFAs then were saponified into fatty acids and both types of lipids were esterified with methanol to free fatty acid methyl esters, as outlined in Frostegård et al. (1991) with modifications by Bischoff et al. (2016). The fatty acid methyl esters were then separated by gas chromatography using an Agilent 7890A GC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a Zebron capillary GC column (60 m, 0.25 mm diameter and 0.25 µm film thickness; Phenomenex, Torrance, California, USA) and quantified with a flame ionization detector, using He as carrier gas. Glyceryl tridodecanoate and nonadecanoic acid were used as internal standards during the extraction and tridecanoic acid methyl ester was added to each sample and standard before analysis as a recovery standard.

Data Analysis

Data from shoot, root P content, shoot N:P ratio, photosynthetic capacity, and LMWOA were subjected to one-way ANOVA analysis. The Duncan post-hoc test was employed to test for differences of mean values ($P<0.05$) between the different P sources offered in the fungal compartment at each time point. Two Spearman correlation tests ($P<0.05$) were carried out. First, between the PLFA biomarkers and the incorporated P (mg/plant); second, between mycorrhizal root status parameters (arbuscules%, vesicles%, mycorrhization%) and those fungal biomarkers that correlated significantly with incorporated P. This was done to determine the possible relationship between the mycorrhizal function at the root level and the P incorporation from the different sources via the mycorrhizal pathway, measured as fungal biomarkers (PLFA and NLFA). For each P source, a principal components analysis (PCA) was conducted on data of the relative abundance of all detected PLFA biomarkers in the fungal compartment, to separate the microbial community into distinct microbial groups. The PCA summarized the correlations among the set of PLFA biomarkers with a smaller set of linear combinations. PCA identifies directions along which the variation in the data is maximal (James et al. 2013). In a second step, the total P incorporated into the plant tissues at each time point was added to the each PCA as a supplementary variable. Total P incorporated values were projected onto the vector subspace generated by the factors having weights of zero in the analysis (Legendre and Gallagher, 2001). The addition of incorporated P as supplementary variable into the PCA was used to identify which PLFA biomarkers were closely related to the incorporation of P from the different sources into the mycorrhizal plant. The PCA represented in biplots illustrate changes in PLFA abundances associated with time (harvesting days) and P incorporation, separately for each P source. The PCA was performed with XLSTAT v.2018 (Adinsoft, 2018) and the ANOVA and correlations tests were performed with SPSS v. 24 (IBM Corporation, 2016).

Results

Phosphorus Content in the Plant Tissues

In the previous Study 2 (Andrino et al., Submitted), we already showed that all mycorrhizal plants with access to a OP, PA, GOE-OP and GOE-PA incorporated P into the plant tissues, but in different amounts and at different rates, while none of the controls (GOE, M+ and M-) showed any P incorporation. All AM plants with a P source in the fungal compartment showed significantly higher P content in the shoots compared to the roots (Figure 2). AM plants that accessed free OP and PA showed a P dilution in their shoot tissue until day 35, then from day 49 onwards the P content in their plant tissues significantly increased until the end of the experiment as compared to all controls (Figure 2A). For treatments offering GOE-PA and GOE-OP the P content in plant shoots was significantly higher from day 77 in comparison to all controls (Figure 2A). As for shoots, the root P content in all treatments showed a dilution. Only the treatments offering OP showed significantly higher P contents in the root tissue at day 91 (Figure 2B).

Shoot N:P Ratios

Mycorrhizal plants with access to free OP and PA suffered from P deficiency from day 21 until day 35, since the N:P ratio was greater than 20 (Figure 3). At day 49 until day 91, AM plants with access to OP and PA became non-P-deficient plants, as indicated by mean N:P values lower than 20. GOE-OP and GOE-PA treatments exhibited P deficiency from day 21 until day 49. At day 77, plants with access to both P forms bound to goethite showed a slight P deficiency by maintaining N:P values close to 20. All controls exhibited a significant P deficiency from day 21 until day 91, compared to all treatments that accessed a P source (Figure 3).

Photosynthetic capacity

Controls showed a significantly lower photosynthetic rate from day 21 until day 91, compared to all treatments that accessed P sources. Mycorrhizal plants accessing free OP and PA exhibited similar photosynthetic capacity until day 35. After day 49, and coinciding with P incorporation into the plant tissues, the photosynthetic rate significantly increased for treatments accessing OP and PA as compared to the controls. Only the treatment containing GOE-OP showed significant differences in photosynthetic rate compared to GOE-PA, M+, and M- until day 77 (Figure 4B). At day 77, P from GOE-PA and GOE-OP had been incorporated and their photosynthetic rate values significantly increased as compared to the controls. At day 91, there were no significant differences in the photosynthetic capacity among all P sources (Figure 4).

Low-Molecular-Weight Organic Acids in the Fungal Compartment

Before and after an AM plant incorporated a given P source, the LMWOAs acetic and gluconic acid exhibited a higher content among the monocarboxylic acids (mono), oxalic acid in the dicarboxylic group (di), and citric acid in the group of tricarboxylic (tri) LMWOAs (Figure 5). Those mesocosms containing free OP in the fungal compartment only showed significant higher content of gluconic (mono) acid before P was incorporated into the plant tissues. The treatment containing PA showed a significantly higher content of acetic (mono), butyric (mono), lactic (mono), and citric (tri) LMWOAs before the AM plant incorporated P. After the AM plant incorporated P only acetic (mono) and citric (tri) LMWOAs were present. The treatment containing GOE-OP exhibited a significant higher content of all monocarboxylic acids, succinic (di), oxalic (di), and citric (tri) LMWOAs before the AM plant incorporated P. After the AM plant incorporated P, gluconic (mono), lactic (mono), malic (di), and oxalic (di) LMWOAs were observed (Figure 5). The fungal compartment containing goethite-bound PA showed a significantly higher content of butyric (mono), gluconic (mono), and citric (tri) acid before the AM plant incorporated P, while after P incorporation acetic (mono), gluconic (mono), lactic (mono), malic (di) and oxalic (di) acid were found in the fungal compartment.

Fatty Acid Biomarkers and Principal Component Analysis

Only 12 out of the 17 fatty acids were measured in all the different fungal compartments. PLFA biomarkers were not detected at day 7 and were not included in the calculation of the PCA analysis. The variance explained by the PCA for the microbial community developed in the treatment containing OP accounted for 67.6% of the total variance (Figure 6A), 61.6% for PA (Figure 6B), 77.03% for GOE-OP (Figure 6C), and 77.96% in case of GOE-PA (Figure 6D). The PCA analysis evidenced shifts in the microbial communities during the experiment for the four P sources offered in the fungal compartment. Most of the harvesting points were distributed along the first principal component (PC1) and the supplementary variable, plant-incorporated P, correlated with some of the PLFA biomarkers along the PC1. The different treatments containing the four P forms showed the same tendency, where the AMF PLFA biomarker 16:1ω5c always correlated with the amount of incorporated P.

In the OP treatment, harvesting time points where no P (nP) had been incorporated into the plant tissues (nP21, nP35, and P49 harvest points) grouped together, in contrast to harvest points P77 and P91, which were placed in the positive side of the PC1. The PLFA biomarkers grouped on the negative side were all bacterial biomarkers (15:0, 17:0, 18:0). Pointing into the opposite direction, the PLFA biomarkers were the gram-negative biomarker 16:1ω7c, the gram-positive i15:0, and two fungal ones, the 16:1ω5c and the 18:1ω7c (Figure 6A).

For the PA treatment harvest points nP21 and nP35 were located on the negative side of PC1 while P49, P77, and P91 were on the positive one (Figure 6B). The PLFAs grouped on the negative side of PC1 were the bacterial 18:0 and the fungal 18:2ω6,9 biomarkers. On the positive side, there were three bacterial gram-positive PLFA biomarkers i15:0, a15:0, and i16:0 along with two fungal biomarkers 16:1ω5c and 18:1ω7c (Figure 6B).

The PCA analysis on the microbial biomarkers for the GOE-OP treatment showed nP21, nP35 and nP49 grouped on the positive side while P77 and P91 on the negative one. The PLFAs grouped on the positive side were mostly PLFA bacterial biomarkers. On the negative side, there were only two PLFA fungal markers, the 16:1ω5c and the 18:1ω7c (Figure 6C).

In case of the GOE-PA treatment, PCA results were similar as for GOE-OP, plus one PLFA gram-positive biomarker (i16:0) along with the 16:1ω5c and the 18:2ω6,9 PLFA fungal biomarkers (Figure 6D).

Spearman Correlation Analysis: Microbial Biomarkers and Incorporated Phosphorus

Correlation analysis between all PLFA biomarkers and the P incorporated into the plant tissues showed AMF PLFA biomarker 16:1ω5c always correlated positively with incorporated P in the whole plant ($P<0.05$) for the four P forms (Table 3). Treatments containing free OP exhibited a positive correlation with two PLFA gram-positive biomarkers (a15:0 and i15:0). The ones containing free PA correlated with general bacterial PLFA biomarkers 14:0, 15:0 and also with three biomarkers representing gram-positive bacteria (i15:0, a15:0, and i16:0). In the

GOE-OP treatment, no bacterial biomarker correlated positively with the P uptake; only two fungal biomarkers showed positive correlation value (16:1ω5c and 18:1ω7c). Phosphorus incorporation in the GOE-PA treatment correlated with i16:0 PLFA, a gram-positive bacteria marker, and two fungal biomarkers (16:1ω5c and 18:2ω6,9; Table 3).

Spearman Correlation Analysis: Fungal Biomarkers, Incorporated Phosphorus, and Mycorrhizal Root Traits

In all tomato roots mycorrhized with *R. irregularis*, we observed an arum-type arbuscular mycorrhizal association characterized by highly branched hyphal termini arbuscules on intercellular hyphae (Saito and Ezawa, 2016). Spearman correlation analysis between fungal PLFA and NLFA biomarkers, the P incorporated into the plant tissues, and mycorrhizal root traits parameters exhibited that the fatty acid 18:1ω9c did not correlate with any mycorrhizal colonization trait for any of the tested P sources, the reason why this data was not included in Table 4. There was a significant positive correlation between the abundance of arbuscules (%) and the P incorporated over time for all P sources offered at fungal compartment. The same positive correlation was found for the 16:1ω5c PLFA and the abundance of arbuscules (%). All mycorrhizal root traits correlated positively with the AM plants accessing the GOE-PA, GOE-OP, and the NLFA 16:1ω5c. Both P sources bound to goethite also showed a significant positive correlation between mycorrhizal root traits and the PLFA and NLFA 18:1ω7c, 18:2ω6,9, respectively (Table 4).

Discussion

We investigated the mechanisms involved in the acquisition of P by an AM plant, where P sources were added either as free compounds or in association with goethite. In the following, we will briefly discuss several aspects linking the response of plants and associated microbial communities to the variable availability of the offered P sources.

Phosphorus Incorporation into the Plant Tissues and its Impact on the Photosynthetic Function

As shown in the Study 2 (Andrino et al. Submitted), we found the different P sources offered in the fungal compartment incorporated into the AM plants. Before any P was incorporated, we observed a dilution in the shoot and root P contents. After P incorporation by AM plants (for OP and PA at day 49; for GOE-OP and GOE-PA at day 77), P was preferentially stored in the shoots for all AM plants. Only plants accessing free OP and PA incorporated P into the shoots to a sufficient level for restoring the plant P content, up to non-deficient levels of >0.1% dry weight (Besford and Maw, 1974)—treatments with GOE-OP and GOE-PA did not restore initial P content at shoot level. Phosphorus is a key limiting nutrient and plants may function with less of it, having a lower tissue P content, or cycle it more efficiently by exhibiting a higher resorption efficiency (Dalling et al., 2016; Rychter et al., 2016). Our results are in accordance with other studies where AM plants preferentially stored P in the shoots and leaves in comparison to non-mycorrhizal plants (Holste et al., 2017; Yang et al., 2014). Under such artificial deficient P conditions, AM plants that accessed the different P sources were able to recycle more efficiently by mobilising it from the roots and older leaves to new and actively growing leaves. Our results confirm the first hypothesis, as OP and PA were incorporated in higher amounts and faster compared to their goethite-bound analogues.

Mycorrhizal plants accessing a P source (as opposed to the mycorrhized control treatments) showed N:P ratios indicating no P deficiency in case of OP and PA (<20) or a slight P deficiency in case of P forms bound to goethite. In addition, treatments with access to P in the fungal compartment showed similar photosynthetic capacity until the end of the experiment, while all controls showed significantly lower photosynthetic capacity. Augé et al. (2016) showed shoot N:P ratios are closely associated with mycorrhizal influence on net photosynthesis. In line with this, photosynthetic capacity was significantly higher where shoot P content (%dw) was significantly higher and N:P ratios were around 20 and below, in comparison to the controls. Our results on higher photosynthetic capacity suggest a higher sink capacity of the mycorrhizal partner compared to the mycorrhizal and non-mycorrhizal control plants not having access to any P source. Among the causes of AM plants exhibiting better photosynthetic activity, stomatal conductance, and transpiration rates are also a higher content of P in their foliage and/or a larger size, relative to non-mycorrhizal plants (Augé et al., 2016). Mycorrhizal benefit is usually greater when plants are P limited (Hoeksema et al., 2010; Johnson et al., 2014), because AM plants show an enhanced photosynthetic P use efficiency (Valentine et al., 2001). Hidaka and Kitayama (2013) proposed that plants can optimize the allocation of P among shoot P fractions for maintaining their productivity and growth and for reducing the demand for P as their adaptation to P-poor soils. Our results confirm that the P mobilisation from the different sources had an impact on the photosynthetic function via an improved photosynthetic P use efficiency, in order to keep constant transfer of photosynthates to the AMF accessing a P source.

Composition of the Microbial Communities Grown in the Fungal Compartment

The PCA biplots revealed a temporal shift of the microbial communities grown in each fungal compartment containing the different P sources. First, we found the presence of bacterial biomarkers before any P was incorporated into the plant tissues. This was followed by the presence of the AMF PLFA biomarker 16:1ω5c, which positively correlated with the P incorporation from each source. With the exception of GOE-OP, all other P sources showed the presence of gram-positive bacterial biomarkers along with PLFA 16:1ω5c, each correlating positively with P incorporation. Our results point towards AMF being associated with specific microbiota, mainly gram-positive bacteria, depending on the P source offered. We inoculated tomato plants with the strain of *R. irregularis* DOAM 197198 not grown in a monoxenic culture, thus carrying the microorganisms naturally associated with its hyphosphere. Recently, P transfer from phytate via AMF with the assistance of phytate-mineralizing bacteria was confirmed by Hara and Saito (2016). They isolated 187 bacteria from the hyphosphere of the cosmopolitan AMF *R. irregularis* DAOM197198 and found *Claroideoglomus etunicatum* able to mineralize phytic acid. Taktek et al. (2015, 2017) and Wang et al. (2016a) also isolated the bacteria closely attached to the hyphosphere of *R. irregularis* DAOM197198. The four studies showed *R. irregularis* hyphal exudates supported the growth and activity of bacteria with high potential for inorganic and organic P mobilisation and mineralization. Selvakumar et al. (2016) also observed that gram-positive bacteria were more closely associated with AMF spore walls and Lecomte et al. (2011) isolated bacteria closely associated with the mycelium of *R. irregularis* grown *in vitro*. As we observed in our study, they mostly isolated gram-positive bacteria (E.g. *Bacillus cereus*, *B. megaterium*, *B. simplex*, *Kocuria rhizophila*, *Microbacterium ginsengisoli* and two gram-negative *Sphingomonas sp.* and *Variovorax paradoxus*). Our results point towards a synergistic interaction between some gram-positive bacteria and the AMF with abilities to mineralize free and mineral-bound PA (after desorption from goethite), as fungal and bacterial PLFA biomarkers increased in parallel to the P incorporation into the plant tissues.

Battini et al. (2016) supported the idea that AMF may establish complex trophic networks with diverse bacterial groups, allowing them to develop various services for the symbiotic fungi, such as biological control of soil-borne diseases, nitrogen fixation, supply of nutrients, and growth factors. For the GOE-OP treatment, PLFA bacterial biomarkers were not detected in parallel to the AMF PLFA biomarker 16:1ω5c, correlating positively with P incorporation. This finding suggests that AMF played an active role in the mobilisation of OP bound to goethite. A possible mechanism used by AMF to desorb P from the surface of goethite is the release of exudates containing LMWOA, as observed by Tawaraya et al. (2006) in the presence of $FePO_4$. For both P forms bound to goethite, the OP and PA had to be desorbed from the goethite by means of LMWOA and then, in contrast to the desorbed OP, the released PA had still to be hydrolyzed through the action of phosphatases. It is likely that these previous steps delayed and reduced the incorporation of P mobilised from GOE-PA and GOE-OP into the plant tissues. Our results, however, support our third hypothesis, that P incorporation into AM plants was always linked to AMF development together with gram-positive bacteria. This was true for treatments offering PA and GOE-PA as P source, but not for treatments offering GOE-OP.

Organic Acid Composition in the Fungal Compartment

We detected the presence of significantly higher concentrations of LMWOA in the fungal compartments containing PA, GOE-OP, and GOE-PA before and after any P was incorporated into the plant tissues. Malic and oxalic acid (di) showed significant greater concentrations in the GOE-OP and GOE-PA treatment during plant P incorporation. In case of citric acid (tri), the trend was the opposite of the dicarboxylic acids, having a significant higher concentration before any P was incorporated in case of GOE-OP and GOE-PA treatments. The nature of AM fungal exudates involved in P mobilisation from organic and inorganic compounds may be diverse and comprise phosphatases, LMWOA, phenolic compounds, protons, and siderophores (Sato et al., 2015). Only two studies have demonstrated LMWOA exudation by AMF into the soil solution, showing that acetate and formiate (Toljander et al., 2007) and citrate and malate (Tawaraya et al., 2006) were part of the hyphal exudates. We also detected three out of the four LMWOA found in the two previous studies in similar concentration as for citric acid (100 nmol/g fungal compartment substrate). The LMWOA detected in the fungal compartments of GOE-OP and GOE-PA treatments belong to the ones with higher chelation capacity. Malate (di), oxalate (di), and citrate (tri) are more effective in mobilising P from goethite or amorphous ferric hydroxides as compared to LMWOA containing one or more α-hydroxyl groups (Clark and Zeto, 2000; Kisa et al., 2007; Muthukumar et al., 2014; Sitta et al., 2012; Thorley et al., 2015). The presence of mono/di/tri LMWOA prior to the incorporation of P into the AM plants refers to a possible mechanism involved in the acquisition of P from mineral-bound sources, where released organic acids weaken and break the bonds between surface-coordinated P forms and structural metal ions before being mobilised by the AM plant. Studies working on the production of organic acids by AMF to desorb OP or PA bound to goethite are lacking, but there are some studies with non-mycorrhized plants and incubation experiments. For example, Parfitt (1979) explained that one of the mechanisms by which OP could be solubilized and made available is through the action of the LMWOA. Parfitt performed several extraction cycles on goethite-bound OP in combination with different LMWOA, resulting in a higher OP desorption when it was incubated in presence of citrate. In a more recent study, Martin et al. (2004) studied the effects of LMWOA which may be released by non-mycorrhizal plant roots, such as citrate, on the desorption of PA and OP bound to goethite. They found a lower amount of PA desorbed from goethite as compared to OP, which was attributed to the strength of chemical bonds and the high negative charge of the complexes, impairing the influence of other cations and/or ligands. Our results on the amount of P incorporated by the plant from PA and both goethite-bound P sources support the findings of Martin et al., as citric acid concentrations rose prior to the incorporation of P by the plant. The presence of the di/tri LMWOA may suggest a mechanism used by AMF with or without the aid of the associated microbiota to desorb P from goethite surfaces. First, a process of desorption took place by means of ligand exchange through the action of LMWOA, such as oxalic, succinic, and citric acid, rendering PA then mineralizable through the action of acid phosphatases secreted by the AMF (Tisserant et al., 2012) or phosphatases from the hyphospheric microbiota, as mentioned above. Some authors (George et al., 2005; Hayes et al., 2000; Otani and Ae, 1999) have pointed out that some LMWOA (E.g. citric acid) have a synergistic effect on the secreted phosphatases (E.g. acid phosphatase), by changing the chemical structure or molecular size of the extracted organic P and making it more accessible to enzymatic action. Accordingly, we found profiles of LMWOAs differing with the accessibilities of the offered P sources. Thereby, LMWOAs with two and three carboxylic groups were more abundant in case of P sources with lower P accessibility. Thus, AMF did more than simply shortening the distance that P ions must diffuse to plant roots, because AM fungi took up not readily available P, suggesting that AM plants could use more insoluble inorganic P forms than non-mycorrhizal plants by means of the production of LMWOA, either by means of the hyphospheric bacteria or the AMF.

Correlation between Mycorrhizal Root Traits and Arbuscular Mycorrhizal Fungi Biomarkers in The Fungal Compartment

Spearman analysis showed a significant positive correlation between three AMF-related PLFA biomarkers, namely 16:1ω5c, 18:1ω7c, and the 18:2ω6,9 and the P incorporation into plant tissues. The composition of *R. irregularis* DAOM 197198 fatty acids ranges from C16:0 to C22:2, with 16:1ω5c as major fatty acid (Calonne et al., 2010). As shown in Table 2, AM fungi *R. irregularis* might contain considerable amounts of other unsaturated fatty acids such as 18:1ω7c, 18:1ω9c and 18:2ω6,9 in the spores, extraradical mycelium, and plant roots. We specifically found the fatty acids 18:1ω7c and 18:2ω6,9 increased in their presence in parallel with fatty acid 16:1ω5c, the common AMF biomarker. Fatty acid 18:2ω6,9 is also known as marker for saprotrophic and ectomycorrhizal fungi (Larsen et al., 1998), but no signs of other saprotrophic fungi were visually detected neither in any fungal compartment, nor in the vicinity of the root system in the plant compartment. Visual microscopic inspection of all root samples did not evidence the presence of intraradical or extraradical septate hyphae.

We described an *Arum*-type of mycorrhization in all the mycorrhized roots. This mycorrhization type is characterized by the presence of arbuscules, which are short-lived structures believed to have a turnover rate of 1 to 2 weeks (Aarle and Olsson, 2003). In the *Arum*-type, arbuscules are the interacting interface between the plant and AMF (Wewer et al., 2014). Phosphorus transfer to the plant occurs in the periarbuscular space of the arbusculated cells (Saito and Ezawa, 2016). This is the main site of C transfer between the root and the hyphae (Dalpé et al., 2012; Kobae et al., 2014; Van Aarle et al., 2005). We observed the abundance of arbuscules (%) positively correlating with P incorporation into the plant and the AMF-related PLFA biomarker 16:1ω5c. This was true for all P sources over the whole runtime of the experiment. Moreover, the fungal biomarker PLFA 18:1ω7c also correlated with the abundance of arbuscules (%) and with the amount of P incorporation from OP and GOE-OP, while the fungal biomarker PLFA 18:2ω6,9 correlated with arbuscules% and P incorporation from GOE-PA. PLFA are vital components of all biological membranes and play a key role in processes such a signal transduction, cytoskeletal rearrangement, membrane trafficking etc. and remain at the place where they were synthesized (Aarle and Olsson, 2003; Dalpé et al., 2012; Debiane et al., 2011). Since AMF only elongates 16C lipid molecules, requiring the plant to produce them (Luginbuehl et al., 2017), the presence of the two unsaturated 18C PLFA fungal biomarkers in our experiment (18:1ω7c and 18:2ω6,9) might support the possibility of a modified hyphae lipid membrane composition as adaptation to the accessibility of the different P sources. Plasticity in fatty acid synthesis attributable to nutritional factors is common in filamentous fungi (Olsson et al., 2002). Based on the correlation data, our results point towards a change in the unsaturation level of AMF membrane lipids with the changing quality of the offered P sources. *R. irregularis*, therefore, might have modified its lipid composition in response to the different P sources. Consequently, the lipid membrane increased its fluidity to keep its integrity compatible with an optimal membrane functionality (Calonne et al. 2010). Membrane fluidity depends on its phospholipid composition of varying length and saturation. Unsaturated lipid chains are more fluid than lipids containing hydrogens and thus have only single bonds. The unsaturated double bonds make it harder for the lipids to pack together by putting kinks into the otherwise straightened hydrocarbon chain (Reichle, 1989). For successful adaptation to altered physicochemical environments, the active remodelling of membrane lipid composition is an essential feature and depends on both strain properties and cultivation conditions (Bentivenga and Morton, 1994; Čertík et al., 2005). Changes in membrane fluidity influence membrane processes such as transport, enzyme activities, and signal transduction (Benyagoub et al., 1996; Turk et al., 2007). For instance, the filamentous fungi *Aspergillus terreus* and *Alternaria alternata* showed an increased content of two unsaturated fatty acids linoleic acid (18:2) and oleic acid (18:1) when the fungi grew on media containing copper ions (Al Abboud and Alawlaqi, 2011). Three *Aureobasidium pullulans* strains were grown in presence of increasing cadmium concentrations. The cell membrane showed an increased fatty acid unsaturation, especially in the levels of linoleic acid (Čertík et al., 2005). A significant increase in membrane fluidity was detected also for ectomycorrhizal fungi *Lactarius piperatus* grown on media supplemented with 1 or 10 mM aluminum for 21 days (Žel et al., 1992). Salt stress also caused an increase in fatty acid unsaturation in the halophilic yeast *Hortaea werneckii* and halotolerant ascomycota *Aureobasidium pullulans*, where the most abundant fatty acids in phospholipids contained C16 and C18 chain lengths with a high content of C18:2 (Turk et al., 2004). When the salt-tolerant yeast *Zygosuccharomyces ro*uxii was grown in 15% NaCl media, the relative amounts of C16:1 and C18:1 fatty acids increased (Hosono, 1992). There are also some reports investigating the effects of benzo[a]pyrene, a polycyclic aromatic hydrocarbon soil contaminant, on chicory roots mycorrhized with *R. irregularis*. For example, Debiane et al. (2012) showed an increased in unsaturated PLFA, principally C16:0, C18:2 and C18:3. They also found the sterol biosynthesis-inhibiting fungicide propiconazole affecting fatty acid composition and proportion, with a significant increase of *R. irregularis* unsaturated fatty acids C18:1, C18:3, C20:2, C22:2 compared to the control. Therefore, it is plausible to consider that the different P sources may have affected the way in which the AMF lipid membrane was organized, by increasing unsaturated lipids in the case the AMF developed on a P source bound to goethite.

Mycorrhizal root traits expressed as abundance of arbuscules (%), vesicles (%), and total mycorrhization intensity (%) positively correlated with AMF-related NLFA biomarkers 16:1ω5c and 18:1ω7c for the GOE-PA treatment and 16:1ω5c, 18:1ω7c, and 18:2ω6,9 for the GOE-OP treatment. These results point to a production of these NLFA by AMF plants mobilising the less accessible P sources, in order to provide the energy for incorporating the goethite-associated P. The required energy was met by higher photosynthetic capacity found in all treatments containing a P source, as the photosynthetic C sink strength of AM fungi is determined by the growth and maintenance of both the intra- and extraradical mycelium (Dalpé et al., 2012; Graham et al., 1995). Photoassimilates are accumulated in the AMF as glycogen and palmitic acid (16C molecules). The latter is incorporated into triacylglycerol (NLFA), the main C reserve of AMF (Bago et al., 2002; Trépanier et al., 2005; Wright et al., 1998). Then, NLFA are stored in intraradical vesicles, spores, and extraradical mycelium to support the translocation of C within the mycelium (Valentine and Kleinert 2007; Kaschuk et al. 2009). Finally, they are metabolized in the mycelium through the glyoxalate cycle to provide the major fungal energy source as respiratory substrate (Aarle and Olsson, 2003; Olsson et al., 2005). In conclusion, our results indicate that there was a greater demand to store energy in the form of NLFA for the treatments where less accessible P sources had to be mobilised. Consistently, the photosynthetic activity had to be increased to support the production and consumption of NLFAs, what we indeed observed.

Conclusions

R. irregularis seems not to be a specialist species in terms of mobilising P bound to goethite, since AM plants did not restore their initial P tissue contents during the time course experiment. This is not surprising, as it is a frequent dweller in agricultural contexts, thus not likely to be a functional specialist. Nevertheless, the use of this cosmopolitan species enabled us to recognize some underlying mechanisms of P mobilisation from the different pools present in soil:

1. As proposed in the first hypothesis, the four P sources offered at the fungal compartment were all mobilised and incorporated into the AM plant, but in different amounts and rates. AM plants preferentially stored P in the shoots, in comparison to non-mycorrhizal plants.
2. As stated in the second hypothesis, we found P mobilisation from the four P sources having a positive influence on the photosynthetic function, through an improved photosynthetic P use efficiency, in comparison to those controls without access to a P source.
3. The third hypothesis was validated as P incorporation in AM plants was linked to the development of the AMF together with gram-positive bacteria for treatments containing PA and GOE-PA, but not for GOE-OP. To our surprise, the putative AMF-related PLFA biomarkers might indicate the different P sources influencing the fungal membrane lipid composition; by increasing unsaturated lipids in the case, the AMF mobilises goethite-associated P. Since NLFA acts as an energy reservoir for AMF, we found the AMF-related NLFA biomarkers increased in parallel with P incorporation and mycorrhization

traits, for those treatments containing GOE-OP and GOE-PA, in order to support the AMF energy demands.

4. As stated in the fourth hypothesis, we found a different profile of LMWOA at each fungal compartment containing the different P sources. Fungal or bacterial LMWOA with two and three carboxylic groups were more abundant before and during P mobilisation from PA, GOE-PA, and GOE-OP, pointing to displacement reactions via ligand exchange as important mechanism to mobilise P.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contribution

JB, GG, AA, and RM designed the experiment. AA prepared the plant and fungal material. AA conducted the experiment and analysed the data. SK analysed the LMWOA on the fungal compartment samples. JB, GG, and RM supervised the research. AA wrote the paper with contributions from JB, GG, RM and SK.

Funding

We thank the German Federal Ministry of Education and Research for the funding of this project in the framework of the DFG-RTG 1798 “Signaling at the Plant-Soil Interface” as well as the DFG priority program SPP 1685 “Forest Strategies for limited Phosphorus Resources”.

Acknowledgments

The authors are thankful for the great help and guidance received by Dr. Leopold Sauheitl, Silke Bokeloh, Elke Eichmann-Prusch, Ulrike Pieper, Heike Steffen, Michael Klatt, and Martin Schrapel. We also want to thank the support received by Dr. Marcus Horn for the LMWOA sample preparation and analysis.

References

Aarle, I. M. Van, and Olsson, P. A. (2003). Fungal Lipid Accumulation and Development of Mycelial Structures by Two Arbuscular Mycorrhizal Fungi Fungal Lipid Accumulation and Development of Mycelial Structures by Two Arbuscular Mycorrhizal Fungi. *Appl.*

Environ. Microbiol. 69, 6762–6767. doi:10.1128/AEM.69.11.6762.

Adinsoft (2018). XLSTAT v.2018. Available at: https://circle.ubc.ca/handle/2429/4644.

Agnolucci, M., Battini, F., Cristani, C., and Giovannetti, M. (2015). Diverse bacterial communities are recruited on spores of different arbuscular mycorrhizal fungal isolates. *Biol. Fertil. Soils* 51, 379–389. doi:10.1007/s00374-014-0989-5.

Al Abboud, M., and Alawlaqi, M. (2011). Biouptake of Copper and Their Impact on Fungal Fatty Acids. *Aust. J. Basic Appl. Sci.* 5, 283–290.

Andrino, A., Boy, J., Mikutta, R., Sauheitl, L., and Guggenberger, G. Carbon investment required for the mobilisation of inorganic and organic phosphorus bound to goethite by an arbuscular mycorrhiza (Solanum lycopersicum x Rhizophagus irregularis). *Front. Environ. Sci.*

Augé, R. M., Toler, H. D., and Saxton, A. M. (2016). Mycorrhizal Stimulation of Leaf Gas Exchange in Relation to Root Colonization, Shoot Size, Leaf Phosphorus and Nitrogen: A Quantitative Analysis of the Literature Using Meta-Regression. *Front. Plant Sci.* 7, 1084. doi:10.3389/fpls.2016.01084.

Bago, B., Zipfel, W., Williams, R. M., Jun, J., Arreola, R., Lammers, P. J., et al. (2002). Translocation and utilization of fungal storage lipid in the arbuscular mycorrhizal symbiosis. *Plant Physiol.* 128, 108–124. doi:10.1104/pp.010466.

Battini, F., Cristani, C., Giovannetti, M., and Agnolucci, M. (2016). Multifunctionality and diversity of culturable bacterial communities strictly associated with spores of the plant beneficial symbiont Rhizophagus intraradices. *Microbiol. Res.* 183, 68–79. doi:10.1016/j.micres.2015.11.012.

Bentivenga, S. P., and Morton, J. B. (1994). Stability and Heritability of Fatty-Acid Methyl-Ester Profiles of Glomalean Endomycorrhizal Fungi. *Mycol. Res.* 98, 1419–1426. doi:10.1016/S0953-7562(09)81073-3.

Benyagoub, M., Willemot, C., and Bélanger, R. R. (1996). Influence of a subinhibitory dose of antifungal atty acids from Sporothrix flocculosa on cellular lipid composition in fungi. *Lipids* 31, 1077–1082. doi:10.1007/BF02522465.

Besford, R. T., and Maw, G. A. (1974). Uptake and distribution of potassium in tomato plants. *Plant Soil* 41, 601–618. doi:10.1007/BF02185819.

Bharadwaj, D. P., Alström, S., and Lundquist, P.-O. O. (2012). Interactions among Glomus irregulare, arbuscular mycorrhizal spore-associated bacteria, and plant pathogens under in vitro conditions. *Mycorrhiza* 22, 437–447. doi:10.1007/s00572-011-0418-7.

Bischoff, N., Mikutta, R., Shibistova, O., Puzanov, A., Reichert, E., Silanteva, M., et al. (2016).

Land-use change under different climatic conditions: Consequences for organic matter and microbial communities in Siberian steppe soils. *Agric. Ecosyst. Environ.* 235, 253–264. doi:10.1016/j.agee.2016.10.022.

Boldt, K., Pörs, Y., Haupt, B., Bitterlich, M., Kühn, C., Grimm, B., et al. (2011). Photochemical processes, carbon assimilation and RNA accumulation of sucrose transporter genes in tomato arbuscular mycorrhiza. *J. Plant Physiol.* 168, 1256–1263. doi:10.1016/j.jplph.2011.01.026.

Bravo, A., Brands, M., Wewer, V., Dörmann, P., and Harrison, M. J. (2017). Arbuscular mycorrhiza-specific enzymes FatM and RAM2 fine-tune lipid biosynthesis to promote development of arbuscular mycorrhiza. *New Phytol.* 214, 1631–1645. doi:10.1111/nph.14533.

Brundrett, M., Bougher, N., Dell, B., Grove, T., and Malajczuk, N. (1996). *Working with mycorrhizas in forestry and agriculture*. Canberra: Australian Centre for International Agricultural Research.

Calonne, M., Fontaine, J., Debiane, D., Laruelle, F., Grandmougin-ferjani, A., and Sahraoui, A. L. (2010). Propiconazole Toxicity on the Non-Target Organism , the Arbuscular Mycorrhizal Fungus, Glomus irregulare. *Fungicides*, 325–346.

Čertík, M., Breierová, E., and Juršíková, P. (2005). Effect of cadmium on lipid composition of Aureobasidium pullulans grown with added extracellular polysaccharides. *Int. Biodeterior. Biodegrad.* 55, 195–202. doi:10.1016/j.ibiod.2004.11.005.

Choudhary, D. K., Varma, A., and Tuteja, N. (2017). “Mycorrhizal Helper Bacteria: Sustainable Approach,” in *Mycorrhiza - Function, Diversity, State of the Art* (Cham: Springer International Publishing), 61–74. doi:10.1007/978-3-319-53064-2_5.

Clark, R. B., and Zeto, S. K. (2000). Mineral acquisition by arbuscular mycorrhizal plants. *J. Plant Nutr.* 23, 867–902. doi:10.1080/01904160009382068.

Dalling, J., Heineman, K., Lopez, O., Wright, S., and Turner, B. (2016). “Nutrient Availability in Tropical Rain Forests: The Paradigm of Phosphorus Limitation,” in *Tropical Tree Physiology* (Springer International Publishing), 261–273. doi:10.1007/978-3-319-27422-5_12.

Dalpé, Y., Trépanier, M., Sahraoui, A. L.-H., Fontaine, J., and Sancholle, M. (2012). Lipids of Mycorrhizas. *Fungal Assoc.* 9, 137–169. doi:10.1007/978-3-642-30826-0_8.

Dames, J. F. (2014). “Biological Control: PGPR and Arbuscular Mycorrhizal Fungi Working Together,” in *Use of Microbes for the Alleviation of Soil Stresses* (New York, NY: Springer New York), 39–53. doi:10.1007/978-1-4939-0721-2_3.

Debiane, D., Calonne, M., Fontaine, J., Laruelle, F., Grandmougin-Ferjani, A., and Lounes-Hadj Sahraoui, A. (2011). Lipid content disturbance in the arbuscular mycorrhizal, Glomus irregulare grown in monoxenic conditions under PAHs pollution. *Fungal Biol.* 115, 782–792. doi:10.1016/j.funbio.2011.06.003.

Debiane, D., Calonne, M., Fontaine, J., Laruelle, F., Grandmougin-Ferjani, A., and Lounès-Hadj Sahraoui, A. (2012). Benzo[a]pyrene induced lipid changes in the monoxenic arbuscular mycorrhizal chicory roots. *J. Hazard. Mater.* 209–210, 18–26. doi:10.1016/j.jhazmat.2011.12.044.

Deubel, A., and Merbach, W. (2005). “Influence of Microorganisms on Phosphorus Bioavailability in Soils,” in *Microorganisms in Soils: Roles in Genesis and Functions* (Berlin/Heidelberg: Springer-Verlag), 177–191. doi:10.1007/3-540-26609-7_9.

Drigo, B., and Donn, S. (2016). “Trading Carbon Between Arbuscular Mycorrhizal Fungi and Their Hyphae-Associated Microbes,” in *Mycorrhizal Mediation of Soil: Fertility, Structure, and Carbon Storage* (Elsevier), 395–412. doi:10.1016/B978-0-12-804312-7.00022-X.

Fernández, I., Merlos, M., López-Ráez, J. A., Martínez-Medina, A., Ferrol, N., Azcón, C., et al. (2014). Defense Related Phytohormones Regulation in Arbuscular Mycorrhizal Symbioses Depends on the Partner Genotypes. *J. Chem. Ecol.* 40, 791–803. doi:10.1007/s10886-014-0473-6.

Field, E., and Marschener, H. (1998). Role of root growth , arbuscular mycorrhiza , and root exudates for the efficiency in nutrient acquisition. *F. Crop. Res.* 56, 203–207. doi:10.1016/S0378-4290(97)00131-7.

Fitter, A. H., Graves, J. D., Watkins, N. K., Robinson, D., and Scrimgeour, C. (1998). Carbon transfer between plants and its control in networks of arbuscular mycorrhizas. *Funct. Ecol.* 12, 406–412. doi:10.1046/j.1365-2435.1998.00206.x.

Frostegård, Å., Tunlid, A., and Bååth, E. (1991). Microbial biomass measured as total lipid phosphate in soils of different organic content. *J. Microbiol. Methods* 14, 151–163. doi:10.1016/0167-7012(91)90018-L.

Geelhoed, J. S., Van Riemsdijk, W. H., and Findenegg, G. R. (1999). Simulation of the effect of citrate exudation from roots on the plant availability of phosphate adsorbed on goethite. *Eur. J. Soil Sci.* 50, 379–390. doi:10.1046/j.1365-2389.1999.00251.x.

George, T. S., Simpson, R. J., Hadobas, P. A., and Richardson, A. E. (2005). Expression of a fungal phytase gene in Nicotiana tabacum improves phosphorus nutrition of plants grown in amended soils. *Plant Biotechnol. J.* 3, 129–140. doi:10.1111/j.1467-

7652.2004.00116.x.

Giasson, P., Jaouich, A., Gagné, S., Moutoglis, P., Gagn, S., and Moutoglis, P. (2005). Arbuscular mycorrhizal fungi involvement in zinc and cadmium speciation change and phytoaccumulation. *Remediat. J.* 15, 75–81. doi:10.1002/rem.20044.

Giles, C. D., Richardson, A. E., Druschel, G. K., and Hill, J. E. (2012). Organic anion-driven solubilization of precipitated and sorbed phytate improves hydrolysis by phytases and bioavailability to Nicotiana tabacum. *Soil Sci.* 177, 591–598. doi:10.1097/SS.0b013e318272f83f.

Giri, B., Giang, P. H., Kumari, R., Prasad, R., and Varma, E. (2005). *Microbial diversity in soils*. Berlin/Heidelberg: Springer-Verlag doi:10.1007/3-540-26609-7_2.

Graham, J. H., Hodge, N. C., Morton, J. B. B., Graham, J. H., and Hodge, N. C. (1995). Fatty Acid methyl ester profiles for characterization of glomalean fungi and their These include : Fatty Acid Methyl Ester Profiles for Characterization of Glomalean Fungi and Their Endomycorrhizae †. *Appl. Environ. Microbiol.* 61, 58–64.

Gui, H., Purahong, W., Hyde, K. D., Xu, J., and Mortimer, P. E. (2017). The arbuscular mycorrhizal fungus Funneliformis mosseae alters bacterial communities in subtropical forest soils during litter decomposition. *Front. Microbiol.* 8, 1120. doi:10.3389/fmicb.2017.01120.

Güsewell, S. (2004). N: P Ratios in Terrestrial Plants: Variation and Functional Significance. *New Phytol.* 164, 243–266. Available at: http://www.jstor.org/stable/1514768.

Hara, S., and Saito, M. (2016). Isolation of Inositol Hexaphosphate (IHP)-Degrading Bacteria from Arbuscular Mycorrhizal Fungal Hyphal Compartments Using a Modified Baiting Method Involving Alginate Beads Containing IHP. *Microbes Environ.* 00, 234–243. doi:10.1264/jsme2.ME15206.

Hayes, J. E. E., Richardson, A. E. E., and Simpson, R. J. J. (2000). Components of organic phosphorus in soil extracts that are hydrolysed by phytase and acid phosphatase. *Biol. Fertil. Soils* 32, 279–286. doi:10.1007/s003740000249.

Hayes, P., Turner, B. L., Lambers, H., and Laliberté, E. (2014). Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient-acquisition strategies along a 2-million-year dune chronosequence. *J. Ecol.* 102, 396–410. doi:10.1111/1365-2745.12196.

He, Z., and Zhu, J. U. N. (1998). Microbial utilization and transformation of phosphate adsorbed by variable charge minerals. *Soil Biol. Biochem.* 30, 917–923. doi:10.1016/S0038-0717(97)00188-0.

Herrera-Medina, M. J., Tamayo, M. I., Vierheilig, H., Ocampo, J. A., and García-Garrido, J.

M. (2008). The jasmonic acid signalling pathway restricts the development of the arbuscular mycorrhizal association in tomato. *J. Plant Growth Regul.* 27, 221–230. doi:10.1007/s00344-008-9049-4.

Hewitt, E. J. (1966). *Sand and water culture methods used in the study of plant nutrition*. Technichal. East Malling , Maidstone, Kent.: University of Bristol Agricultural and Horticultural Research Station, Long Ashton, Bristol.

Hidaka, A., and Kitayama, K. (2013). Relationship between photosynthetic phosphorus-use efficiency and foliar phosphorus fractions in tropical tree species. *Ecol. Evol.* 3, 4872–4880. doi:10.1002/ece3.861.

Hoberg, E., Marschner, P., and Lieberei, R. (2005). Organic acid exudation and pH changes by Gordonia sp. and Pseudomonas fluorescens grown with P adsorbed to goethite. *Microbiol. Res.* 160, 177–187. doi:10.1016/j.micres.2005.01.003.

Hoeksema, J. D., Chaudhary, V. B., Gehring, C. A., Johnson, N. C., Karst, J., Koide, R. T., et al. (2010). A meta-analysis of context-dependency in plant response to inoculation with mycorrhizal fungi. *Ecol. Lett.* 13, 394–407. doi:10.1111/j.1461-0248.2009.01430.x.

Holste, E. K., Kobe, R. K., and Gehring, C. A. (2017). Plant species differ in early seedling growth and tissue nutrient responses to arbuscular and ectomycorrhizal fungi. *Mycorrhiza* 27, 211–223. doi:10.1007/s00572-016-0744-x.

Hosono, K. (1992). Effect of salt stress on lipid composition and membrane fluidity of the salt-tolerant yeast Zygosaccharomyces rouxii. *J. Gen. Microbiol.*, 91–96. doi:10.1099/00221287-138-1-91.

Hwangbo, H., Park, R. D., Kim, Y. W., Rim, Y. S., Park, K. H., Kim, T. H., et al. (2003). 2-Ketogluconic acid production and phosphate solubilization by Enterobacter intermedium. *Curr. Microbiol.* 47, 87–92. doi:10.1007/s00284-002-3951-y.

IBM Corporation (2016). IBM SPSS Statistics for Windows, Version 24.0. *2016*.

Igual, J. M., Valverde, A., Cervantes, E., and Velázquez, E. (2001). Phosphate-solubilizing bacteria as inoculants for agriculture: use of updated molecular techniques in their study. *Agronomie* 21, 561–568. doi:10.1051/agro:2001145.

Jansa, J., Bukovská, P., and Gryndler, M. (2013). Mycorrhizal hyphae as ecological niche for highly specialized hypersymbionts – or just soil free-riders? *Front. Plant Sci.* 4, 134. doi:10.3389/fpls.2013.00134.

Jiang, Y., Wang, W., Xie, Q., Liu, N., Liu, L., Wang, D., et al. (2017). Plants transfer lipids to sustain colonization by mutualistic mycorrhizal and parasitic fungi. *Science (80-.).* 356, 1172–1173. doi:10.1126/science.aam9970.

Johansen, A., Finlay, R. D., and Olsson, P. A. (1996). Nitrogen metabolism of external hyphae of the arbuscular mycorrhizal fungus Glomus intraradices. *New Phytol.* 133, 705–712. doi:10.1111/j.1469-8137.1996.tb01939.x.

Johnson, N. C., Wilson, G. W. T., Wilson, J. A., Miller, R. M., and Bowker, M. A. (2014). Mycorrhizal phenotypes and the Law of the Minimum. *New Phytol.* 205, 1473–1484. doi:10.1111/nph.13172.

Joner, E. J., Van Aarle, I. M., and Vosatka, M. (2000). Phosphatase activity of extra-radical arbuscular mycorrhizal hyphae: A review. in *Plant and Soil*, 199–210. doi:10.1023/A:1026582207192.

Jones, D. L. (1998). Organic acids in the rhizosphere - A critical review. *Plant Soil* 205, 25–44. doi:10.1023/A:1004356007312.

Kaiser, C., Kilburn, M. R., Clode, P. L., Fuchslueger, L., Koranda, M., Cliff, J. B., et al. (2015). Exploring the transfer of recent plant photosynthates to soil microbes: Mycorrhizal pathway vs direct root exudation. *New Phytol.* 205, 1537–1551. doi:10.1111/nph.13138.

Kaschuk, G., Kuyper, T. W., Leffelaar, P. A., Hungria, M., and Giller, K. E. (2009). Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? *Soil Biol. Biochem.* 41, 1233–1244. doi:http://dx.doi.org/10.1016/j.soilbio.2009.03.005.

Keymer, A., Pimprikar, P., Wewer, V., Huber, C., Brands, M., Bucerius, S. L., et al. (2017). Lipid transfer from plants to arbuscular mycorrhiza fungi. *Elife* 6. doi:10.7554/eLife.29107.

Kisa, M., Sanon, A., Thioulouse, J., Assigbetse, K., Sylla, S., Spichiger, R., et al. (2007). Arbuscular mycorrhizal symbiosis can counterbalance the negative influence of the exotic tree species Eucalyptus camaldulensis on the structure and functioning of soil microbial communities in a sahelian soil. *FEMS Microbiol. Ecol.* 62, 32–44. doi:10.1111/j.1574-6941.2007.00363.x.

Kobae, Y., Gutjahr, C., Paszkowski, U., Kojima, T., Fujiwara, T., and Hata, S. (2014). Lipid droplets of arbuscular mycorrhizal fungi emerge in concert with arbuscule collapse. *Plant Cell Physiol.* 55, 1945–1953. doi:10.1093/pcp/pcu123.

Koerselman, W., and Meuleman, A. (1996). The vegetation N: P ratio: a new tool to detect the nature of nutrient limitation. *J. Appl. Ecol.* Available at: http://www.jstor.org/stable/2404783 [Accessed December 10, 2014].

Köhl, L., Lukasiewicz, C. E., and Van der Heijden, M. G. A. (2016). Establishment and effectiveness of inoculated arbuscular mycorrhizal fungi in agricultural soils. *Plant Cell*

Environ. 39, 136–146. doi:10.1111/pce.12600.

Koide, R. T., and Kabir, Z. (2000). Extraradical hyphae of the mycorrhizal fungus Glomus intraradices can hydrolyse inorganic phosphate. *New Phytol.* 148, 511–517. doi:10.1046/j.1469-8137.2000.00776.x.

Krishnamoorthy, R., Kim, K., Subramanian, P., Senthilkumar, M., Anandham, R., and Sa, T. (2016). Arbuscular mycorrhizal fungi and associated bacteria isolated from salt-affected soil enhances the tolerance of maize to salinity in coastal reclamation soil. *Agric. Ecosyst. Environ.* 231, 233–239. doi:10.1016/j.agee.2016.05.037.

Larsen, J., Olsson, P. A., and Jakobsen, I. (1998). The use of fatty acid signatures to study mycelial interactions between the arbuscular mycorrhizal fungus Glomus intraradices and the saprotrophic fungus Fusarium culmorum in root-free soil. *Mycol. Res.* 102, 1491–1496. doi:10.1017/S0953756298006558.

Lecomte, J., St-Arnaud, M., and Hijri, M. (2011). Isolation and identification of soil bacteria growing at the expense of arbuscular mycorrhizal fungi. *FEMS Microbiol. Lett.* 317, 43–51. doi:10.1111/j.1574-6968.2011.02209.x.

Legendre, P., and Gallagher, E. D. (2001). Ecologically meaningful transformations for ordination of species data. *Oecologia* 129, 271–280. doi:10.1007/s004420100716.

Luginbuehl, L. H., Menard, G. N., Kurup, S., Van Erp, H., Radhakrishnan, G. V, Breakspear, A., et al. (2017). Fatty acids in arbuscular mycorrhizal fungi are synthesized by the host plant. *Science* 356, 1175–1178. doi:10.1126/science.aan0081.

Maathuis, F. J. (2009). Physiological functions of mineral macronutrients. *Curr. Opin. Plant Biol.* 12, 250–258. doi:10.1016/J.PBI.2009.04.003.

Mäder, P., Vierheilig, H., Alt, M., and Wiemken, A. (1993). Boundaries between soil compartments formed by microporous hydrophobic membranes (GORE-TEXR) can be crossed by vesicular-arbuscular mycorrizal fungi but not by ions in the soil solution. *Plant Soil* 152, 201–206. doi:10.1007/BF00029089.

Mäder, P., Vierheilig, H., Streitwolf-Engel, R., Boller, T., Frey, B., Christie, P., et al. (2000). Transport of 15N from a soil compartment separated by a polytetrafluoroethylene membrane to plant roots via the hyphae of arbuscular mycorrhizal fungi. *New Phytol.* 146, 155–161. doi:10.1046/j.1469-8137.2000.00615.x.

Manaut, N., Sanguin, H., Ouahmane, L., Bressan, M., Thioulouse, J., Baudoin, E., et al. (2014). Potentialities of ecological engineering strategy based on native arbuscular mycorrhizal community for improving afforestation programs with carob trees in degraded environments. *Ecol. Eng.* 79, 113–119. doi:10.1016/j.ecoleng.2015.03.007.

Manchanda, G., Singh, R. P., Li, Z. F., and Zhang, J. J. (2017). "Mycorrhiza: Creating Good Spaces for Interactions," in *Mycorrhiza - Function, Diversity, State of the Art* (Cham: Springer International Publishing), 39–60. doi:10.1007/978-3-319-53064-2_4.

Martin, M., Celi, L., and Barberis, E. (2004). Desorption and Plant Availability of Myo-Inositol Hexaphosphate Adsorbed on Goethite. *Soil Sci.* 169, 115–124. doi:10.1097/01.ss.0000117787.98510.9d.

McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., and Swan, J. A. (1990). A New Method which Gives an Objective Measure of Colonization of Roots by Vesicular-Arbuscular Mycorrhizal Fungi. *New Phytol.* 115, 495–501. doi:10.1111/j.1469-8137.1990.tb00476.x.

Mishra, V., Gupta, A., Kaur, P., Singh, S., Singh, N., Gehlot, P., et al. (2016). Synergistic effects of Arbuscular mycorrhizal fungi and plant growth promoting rhizobacteria in bioremediation of iron contaminated soils. *Int. J. Phytoremediation* 18, 697–703. doi:10.1080/15226514.2015.1131231.

Morcillo, R. J. L., Navarrete, M. I. T., Bote, J. A. O., Monguio, S. P., and García-Garrido, J. M. (2016). Suppression of allene oxide synthase 3 in potato increases degree of arbuscular mycorrhizal fungal colonization. *J. Plant Physiol.* 190, 15–25. doi:10.1016/j.jplph.2015.11.003.

Muthukumar, T., Priyadharsini, P., Uma, E., Jaison, S., and Pandey, R. R. (2014). "Role of Arbuscular Mycorrhizal Fungi in Alleviation of Acidity Stress on Plant Growth," in *Use of Microbes for the Alleviation of Soil Stresses, Volume 1* (New York, NY: Springer New York), 43–71. doi:10.1007/978-1-4614-9466-9_3.

Nadal, M., and Paszkowski, U. (2013). Polyphony in the rhizosphere: Presymbiotic communication in arbuscular mycorrhizal symbiosis. *Curr. Opin. Plant Biol.* 16, 473–479. doi:http://dx.doi.org/10.1016/j.pbi.2013.06.005.

Nash, D. M., Haygarth, P. M., Turner, B. L., Condron, L. M., McDowell, R. W., Richardson, A. E., et al. (2014). Using organic phosphorus to sustain pasture productivity: A perspective. *Geoderma* 221–222, 11–19. doi:10.1016/j.geoderma.2013.12.004.

Nuccio, E. E., Hodge, A., Pett-Ridge, J., Herman, D. J., Weber, P. K., and Firestone, M. K. (2013). An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. *Environ. Microbiol.* 15, 1870–1881. doi:10.1111/1462-2920.12081.

Oberson, A., Friesen, D. K., Rao, I. M., Bühler, S., Frossard, E., Buhler, S., et al. (2001). Phosphorus transformations in an Oxisol under contrasting land-use systems: The role of the soil microbial biomass. in *Plant and Soil* (Kluwer Academic Publishers), 197–210. doi:10.1023/A:1013301716913.

Ognalaga, M., Frossard, E., and Thomas, F. (1994). Glucose-1-phosphate and myo-inositol hexaphosphate adsorption mechanisms on goethite. *Soil Sci. Soc. Am. J.* 58, 332–337. doi:10.2136/sssaj1994.03615995005800020011x.

Olsson, P. A., Aarle, I. M. Van, Gavito, M. E., Bengtson, P., and Bengtson, G. (2005). 13 C Incorporation into Signature Fatty Acids as an Assay for Carbon Allocation in Arbuscular Mycorrhiza C Incorporation into Signature Fatty Acids as an Assay for Carbon Allocation in Arbuscular Mycorrhiza. *Appl. Environ. Microbiol.* 71, 2592–2599. doi:10.1128/AEM.71.5.2592.

Olsson, P. A., and Johansen, A. (2000). Lipid and fatty acid composition of hyphae and spores of arbuscular mycorrhizal fungi at different growth stages. *Mycol. Res.* 104, 429–434. doi:10.1017/S0953756299001410.

Olsson, P. A., van Aarle, I. M., Allaway, W. G., Ashford, A. E. A. E., and Rouhier, H. (2002). Phosphorus Effects on Metabolic Processes in Monoxenic Arbuscular Mycorrhiza Cultures. *Plant Physiol.* 130, 1162–1171. doi:10.1104/pp.009639.

Ordoñez, Y. M., Fernandez, B. R., Lara, L. S., Rodriguez, A., Uribe-Vélez, D., and Sanders, I. R. (2016). Bacteria with phosphate solubilizing capacity alter mycorrhizal fungal growth both inside and outside the root and in the presence of native microbial communities. *PLoS One* 11, 1–18. doi:10.1371/journal.pone.0154438.

Otani, T., and Ae, N. (1999). Extraction of organic phosphorus in Andosols by various methods. *Soil Sci. Plant Nutr.* 45, 151–161. doi:10.1080/00380768.1999.10409331.

Owen, D., Williams, A. P., Griffith, G. W., and Withers, P. J. A. (2015). Use of commercial bio-inoculants to increase agricultural production through improved phosphrous acquisition. *Appl. Soil Ecol.* 86, 41–54. doi:10.1016/j.apsoil.2014.09.012.

Parfitt, R. L. (1979). The availability of P from phosphate-goethite bridging complexes. Desorption and uptake by ryegrass. *Plant Soil* 53, 55–65. doi:10.1007/BF02181879.

Paterson, E., Sim, A., Davidson, J., and Daniell, T. J. (2016). Arbuscular mycorrhizal hyphae promote priming of native soil organic matter mineralisation. *Plant Soil* 408, 243–254. doi:10.1007/s11104-016-2928-8.

Rakshit, A., and Bhadoria, pratapbhanu (2010). Role of VAM on growth and phosphorus nutrition of maize with low soluble phosphate fertilization. *Acta Agronómica* 59, 119–123. Available at: http://www.revistas.unal.edu.co/index.php/acta_agronomica/article/view/14101/14960.

Reichle, D. E. (1989). “Biomembranes: Molecular Structure and Function,” in *Springer Advanced Texts in Chemistry* (University of California Press), 533. doi:10.1007/978-1-4757-2065-5.

Rich, M. K., Nouri, E., Courty, P. E., and Reinhardt, D. (2017). Diet of Arbuscular Mycorrhizal Fungi: Bread and Butter? *Trends Plant Sci.* 22, 652–660. doi:10.1016/j.tplants.2017.05.008.

Richardson, A. E. (2001). Prospects for using soil microorganisms to improve the acquisition of phosphorus by plants. *Aust. J. Plant Physiol.* 28, 897–906. doi:10.1071/PP01093.

Ros, M. B. H., De Deyn, G. B., Koopmans, G. F., Oenema, O., and van Groenigen, J. W. (2018). What root traits determine grass resistance to phosphorus deficiency in production grassland? *J. Plant Nutr. Soil Sci.* doi:10.1002/jpln.201700093.

Rychter, A., Rao, I., and Cardoso, J. (2016). “Role of Phosphorus in Photosynthetic Carbon Assimilation and Partitioning,” in *Handbook of Photosynthesis,* 603–625. doi:10.1201/b19498-45.

Saito, K., and Ezawa, T. (2016). “Phosphorus metabolism and transport in arbuscular mycorrhizal symbiosis,” in *Molecular Mycorrhizal Symbiosis* (Hoboken, NJ, USA: John Wiley & Sons, Inc.), 197–216. doi:10.1002/9781118951446.ch12.

Sato, T., Ezawa, T., Cheng, W., and Tawaraya, K. (2015). Release of acid phosphatase from extraradical hyphae of arbuscular mycorrhizal fungus Rhizophagus clarus. *Soil Sci. Plant Nutr.* 61, 269–274. doi:10.1080/00380768.2014.993298.

Schachtman, D. P., Reid, R. J., and Ayling, S. M. (1998). Update on Phosphorus Uptake Phosphorus Uptake by Plants: From Soil to Cell. 447–453. doi:10.1104/pp.116.2.447.

Scheublin, T. R., Sanders, I. R., Keel, C., and van der Meer, J. R. (2010). Characterisation of microbial communities colonising the hyphal surfaces of arbuscular mycorrhizal fungi. *ISME J.* 4, 752–763. doi:10.1038/ismej.2010.5.

Selvakumar, G., Krishnamoorthy, R., Kim, K., and Sa, T. M. (2016). Genetic diversity and association characters of bacteria isolated from arbuscular mycorrhizal fungal spore walls. *PLoS One* 11, 1–16. doi:10.1371/journal.pone.0160356.

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., et al. (2011). Phosphorus Dynamics: From Soil to Plant. *Plant Physiol.* 156, 997–1005. doi:10.1104/pp.111.175232.

Sitta, N., Davoli, P., Zambonelli, A., Iotti, M., Boutahir, S., Lancellotti, E., et al. (2012). Edible Ectomycorrhizal Mushrooms. *Mycorrhiza* 23, 1096–1099. doi:10.5897/SRE2013.5529.

Smith, S. E., Smith, F. A., and Jakobsen, I. (2003). Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiol.* 133, 16–20.

doi:10.1104/pp.103.024380.

Taktek, S., St-Arnaud, M., Piché, Y., Fortin, J. A., and Antoun, H. (2017). Igneous phosphate rock solubilization by biofilm-forming mycorrhizobacteria and hyphobacteria associated with Rhizoglomus irregulare DAOM 197198. *Mycorrhiza* 27, 13–22. doi:10.1007/s00572-016-0726-z.

Taktek, S., Trépanier, M., Servin, P. M., St-Arnaud, M., Piché, Y., Fortin, J.-A. A., et al. (2015). Trapping of phosphate solubilizing bacteria on hyphae of the arbuscular mycorrhizal fungus Rhizophagus irregularis DAOM 197198. *Soil Biol. Biochem.* 90, 1–9. doi:10.1016/j.soilbio.2015.07.016.

Tani, M., and Higashi, T. (1999). Vertical distribution of low molecular weight aliphatic carboxylic acids in some forest soils of Japan. *Eur. J. Soil Sci.* 50, 217–226. doi:10.1046/j.1365-2389.1999.00228.x.

Tarafdar, J. C., and Marschner, H. (1994a). Efficiency of VAM hyphae in utilisation of organic phosphorus by wheat plants. *Soil Sci. Plant Nutr.* 40, 593–600. doi:10.1080/00380768.1994.10414298.

Tarafdar, J. C., and Marschner, H. (1994b). Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. *Soil Biol. Biochem.* 26, 387–395. doi:10.1016/0038-0717(94)90288-7.

Tarafdar, J. C., Yadav, R. S., and Niwas, R. (2002). Relative efficiency of fungal intra- and extracellular phosphatases and phytase. *J. Plant Nutr. Soil Sci.* 165, 17–19. doi:10.1002/1522-2624(200202)165:1<17::AID-JPLN17>3.0.CO;2-C.

Tawaraya, K., Naito, M., and Wagatsuma, T. (2006). Solubilization of insoluble inorganic phosphate by hyphal exudates of arbuscular mycorrhizal fungi. *J. Plant Nutr.* 29, 657–665. doi:10.1080/01904160600564428.

Thorley, R. M. S., Taylor, L. L., Banwart, S. A., Leake, J. R., and Beerling, D. J. (2015). The role of forest trees and their mycorrhizal fungi in carbonate rock weathering and its significance for global carbon cycling. *Plant. Cell Environ.* 38, 1947–1961. doi:10.1111/pce.12444.

Tienebo, E.-O. (2016). Effect of Inoculation of Pseudomonas Chlororaphis 30-84 and / or Rhizophagus Irregularis DAOM 197198 on Maize Anthracnose Leaf Blight and Tomato Zebra Chip Disease. Available at: https://oaktrust.library.tamu.edu/handle/1969.1/158069 [Accessed September 15, 2018].

Tisserant, E., Kohler, A., Dozolme-Seddas, P., Balestrini, R., Benabdellah, K., Colard, A., et al. (2012). The transcriptome of the arbuscular mycorrhizal fungus Glomus intraradices

(DAOM 197198) reveals functional tradeoffs in an obligate symbiont. *New Phytol.* 193, 755–769. doi:10.1111/j.1469-8137.2011.03948.x.

Toljander, J. F., Lindahl, B. D. B. D., Paul, L. R., Elfstrand, M., and Finlay, R. D. (2007). Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. *FEMS Microbiol. Ecol.* 61, 295–304. doi:10.1111/j.1574-6941.2007.00337.x.

Trépanier, M., Bécard, G., Moutoglis, P., Willemot, C., Gagné, S., Avis, T. J., et al. (2005). Dependence of Arbuscular-Mycorrhizal Fungi on Their Plant Host for Palmitic Acid Synthesis Dependence of Arbuscular-Mycorrhizal Fungi on Their Plant Host for Palmitic Acid Synthesis. *Society* 71, 5341–5347. doi:10.1128/AEM.71.9.5341.

Tuomi, J., Kytoviita, M.-M. M., and Hardling, R. (2001). Cost efficiency of nutrient acquisition and the advantage of mycorrhizal symbiosis for the host plant. *Oikos* 92, 62–70. doi:10.1034/j.1600-0706.2001.920108.x.

Turk, M., Abramović, Z., Plemenitaš, A., and Gunde-Cimerman, N. (2007). Salt stress and plasma-membrane fluidity in selected extremophilic yeasts and yeast-like fungi. *FEMS Yeast Res.* 7, 550–557. doi:10.1111/j.1567-1364.2007.00209.x.

Turk, M., Méjanelle, L., Šentjurc, M., Grimalt, J. O., Gunde-Cimerman, N., and Plemenitaš, A. (2004). Salt-induced changes in lipid composition and membrane fluidity of halophilic yeast-like melanized fungi. *Extremophiles* 8, 53–61. doi:10.1007/s00792-003-0360-5.

Turner, B. L. (2008). Resource partitioning for soil phosphorus: A hypothesis. *J. Ecol.* 96, 698–702. doi:10.1111/j.1365-2745.2008.01384.x.

Valentine, A. J., and Kleinert, A. (2007). Respiratory responses of arbuscular mycorrhizal roots to short-term alleviation of P deficiency. *Mycorrhiza* 17, 137–143. doi:10.1007/s00572-006-0093-2.

Valentine, A. J., Osborne, B. A., and Mitchell, D. T. (2001). Interactions between phosphorus supply and total nutrient availability on mycorrhizal colonization, growth and photosynthesis of cucumber. *Sci. Hortic. (Amsterdam).* 88, 177–189. doi:10.1016/S0304-4238(00)00205-3.

Van Aarle, I. M., Cavagnaro, T. R., Smith, S. E., Smith, F. A., and Dickson, S. (2005). Metabolic activity of Glomus intraradices in Arum- and Paris-type arbuscular mycorrhizal colonization. *New Phytol.* 166, 611–618. doi:10.1111/j.1469-8137.2005.01340.x.

Vierheilig, H., Alt-Hug, M., Engel-Streitwolf, R., Mäder, P., and Wiemken, A. (1998a). Studies on the attractional effect of root exudates on hyphal growth of an arbuscular mycorrhizal fungus in a soil compartment-membrane system. *Plant Soil* 203, 137–144.

doi:10.1023/A:1004329919005.

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

Virant-Klun, I., and Gogala, N. (1995). Impact of VAM on phosphorus nutrition of maize with low soluble phosphate fertilization. *J. Plant Nutr.* 18, 1815–1823. doi:10.1080/01904169509365025.

Wang, F., Shi, N., Jiang, R., Zhang, F., and Feng, G. (2016). In situ stable isotope probing of phosphate-solubilizing bacteria in the hyphosphere. *J. Exp. Bot.* 67, 1689–1701. doi:10.1093/jxb/erv561.

Watkins, N. K., Fitter, A. H., Graves, J. D., and Robinson, D. (1996). Carbon transfer between C3 and C4 plants linked by a common mycorrhizal network, quantified using stable carbon isotopes. *Soil Biol. Biochem.* 28, 471–477. doi:http://dx.doi.org/10.1016/0038-0717(95)00189-1.

Wewer, V., Brands, M., and Dörmann, P. (2014). Fatty acid synthesis and lipid metabolism in the obligate biotrophic fungus Rhizophagus irregularis during mycorrhization of Lotus japonicus. *Plant J.* 79, 398–412. doi:10.1111/tpj.12566.

Wright, D. P., Read, D. J., and Scholes, J. D. (1998). Mycorrhizal sink strength influences whole plant carbon balance of Trifofium repens L. *Plant, Cell Environ.* 21, 881–891.

Xu, G. H., Chague, V., Melamed-Bessudo, C., Kapulnik, Y., Jain, A., Raghothama, K. G., et al. (2007). Functional characterization of LePT4: A phosphate transporter in tomato with mycorrhiza-enhanced expression. *J. Exp. Bot.* 58, 2491–2501. doi:10.1093/jxb/erm096.

Yang, Y., Tang, M., Sulpice, R., Chen, H., Tian, S., and Ban, Y. (2014). Arbuscular Mycorrhizal Fungi Alter Fractal Dimension Characteristics of Robinia pseudoacacia L. Seedlings Through Regulating Plant Growth, Leaf Water Status, Photosynthesis, and Nutrient Concentration Under Drought Stress. *J. Plant Growth Regul.* 33, 612–625. doi:10.1007/s00344-013-9410-0.

Žel, J., Schara, M., Svetek, J., Nemec, M., and Gogala, N. (1992). Influence of aluminum on the membranes of mycorrhizal fungi. *Water, Air, Soil Pollut.* 71, 101–109. doi:10.1007/BF00475514.

Zelles, L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biol. Fertil. Soils* 29, 111–129. doi:10.1007/s003740050533.

Zhang, L., Fan, J., Ding, X., He, X., Zhang, F., and Feng, G. (2014). Hyphosphere interactions between an arbuscular mycorrhizal fungus and a phosphate solubilizing bacterium promote phytate mineralization in soil. *Soil Biol. Biochem.* 74, 177–183. doi:10.1016/j.soilbio.2014.03.004.

Zhang, L., Xu, M., Liu, Y., Zhang, F., Hodge, A., and Feng, G. (2016). Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. *New Phytol.* 210, 1022–1032. doi:10.1111/nph.13838.

Table 1. Description of the treatments tested during the time course experiment.

Codes	**Treatment**	**Phosphorus content at the fungal compartment**
M+	Control: Treatment containing an arbuscular mycorrhizal (AM) plant and no phosphorus (P) source at the fungal compartment (FC)	Quartz sand (60 g) + MilliQ water (16 ml)
M-	Control: Non-AM plant and no P source at the FC	Quartz sand (60 g) + MilliQ water (16 ml)
GOE	Control: AM plant and no P source at the FC	Bayferrox 920 Z goethite (24.3 g) + MilliQ water (28ml)
PA	AM plant and phytic acid ($C_6H_{18}O_{24}P_6 \cdot xNa^+ \cdot yH_2O$) as P source at the FC	Quartz sand (60 g) + Solution containing 30 mg P (16 ml)
OP	AM plant and phytic acid (KH_2PO_4) as P source at the FC	Quartz sand (60 g) + Liquid solution containing 30 mg P (16 ml)
GOE-PA	AM plant and phytic acid bound to goethite adsorption complex (1.79 g P/kg.) as P source at the FC	GOE-PA (16.7 g) containing 30 mg P + Bayferrox 920 Z goethite (7.6 g) + MilliQ water (28 ml)
GOE-OP	AM plant and orthophosphate bound to goethite adsorption complex (1.24 g P/kg.) as P source at the FC	GOE-OP (24.3 g) containing 30 mg P + MilliQ water (28ml)

Table 2. List of analysed fatty acid methyl esters (FAME) biomarkers and their microbial group designation.

FAME Biomarker	Microbial group designation (MGD) (Willers et al. 2015)	Presence in AM fungi
10Me16:1	Actinomycetes (A)	none
16:1ω5c	Fungal marker (F)	(Olsson et al. 1995, 1997, 2002; Johansen et al. 1996; Larsen et al. 1998; Green et al. 1999; Olsson and Wilhelmsson 2000; Madan et al. 2002; Aarle and Olsson 2003; Stumpe et al. 2005)
18:1ω7c		
18:1ω9c		
18:2ω6,9		
14:0	General bacterial marker (B)	none
15:0		
17:0		
18:0		
16:1ω7c	Gram-negative bacteria (B-)	none
cy19:0		
a15:0	Gram-positive bacteria (B+)	none
a17:0		
i15:0		
i16:0		
i17:0		
20:4ω6c	Protozoa and nematodes (PN)	none

Table 3. Heatmap showing the Spearman correlations between the PLFA biomarkers and the incorporated P (mg/plant). Designations are as follows: (B) represents general bacterial marker, (B+) gram-positive bacteria, and (F) fungal marker, respectively. Correlation values in bold are significant (* p <0.05, ** p <0.01).

		Incorporated P (mg/plant)			
Biomarker	**Microbial group designation**	**PA**	**OP**	**GOE-PA**	**GOE-OP**
14:00	B	**0.5256***	0.0922		
15:00		**0.5348***	0.1143		
i15:0	B+	**0.7303****	**0.6197***		
a15:0		**0.5219***	**0.6768****		
i16:0		**0.5846***	0.19	0.6868	
16:1ω5c	F	**0.6861****	**0.6621****	**0.7803****	**0.7373****
18:2ω6.9				**0.5953***	
18:1ω9c			0.1033		
18:1ω7c		0.0738	0.3578		**0.7978****

Legend:

0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1

Table 4. Heatmap showing the Spearman correlations between mycorrhizal root status parameters and fungal biomarkers (%) at the fungal compartment. Correlation values in bold are significant (* p <0.05, ** p <0.01, *** p <0.0001). PLFA, phospholipid fatty acid; NLFA, neutral lipid fatty acid; OP, orthophosphate; PA, phytic acid; GOE-PA, phytic acid bound to goethite; GOE-OP, orthophosphate bound to goethite; A, arbuscules; V, vesicles; M, mycorrhization; Inc. P, incorporated P.

			PLFA (%)			**NLFA (%)**		
		Inc. P (mg)	**16:1ω5c**	**18:2ω6,9**	**18:1ω7c**	**16:1ω5c**	**18:2ω6,9**	**18:1ω7c**
PA	A%	**0.771****	**0.548***			0.414	0.121	0.380
	V%	0.429	0.396	0.052		0.432	0.439	**0.53***
	M%	**0.533****	0.430			0.441	0.357	**0.522***
OP	A%	**0.711****	**0.565***		**0.621***	0.342	**0.547***	0.469
	V%	**0.444**	0.116		0.322	0.156	0.184	0.226
	M%	**0.664****	0.430		**0.544***	0.279	0.424	0.412
GOE-PA	A%	**0.56***	**0.774****	**0.597***		**0.815****	0.483	**0.801****
	V%	**0.54***	**0.691****	**0.633***		**0.809****	0.439	**0.82****
	M%	**0.654***	**0.743****	**0.628***		**0.78****	0.478	**0.787****
GOE-OP	A%	**0.62***	**0.587***	0.133	**0.637***	**0.938*****	**0.767****	**0.932*****
	V%	0.008	0.267	0.258	0.206	**0.838*****	0.479	**0.779****
	M%	0.218	0.372	0.189	0.400	**0.91*****	**0.599***	**0.868*****

Legend:

0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1

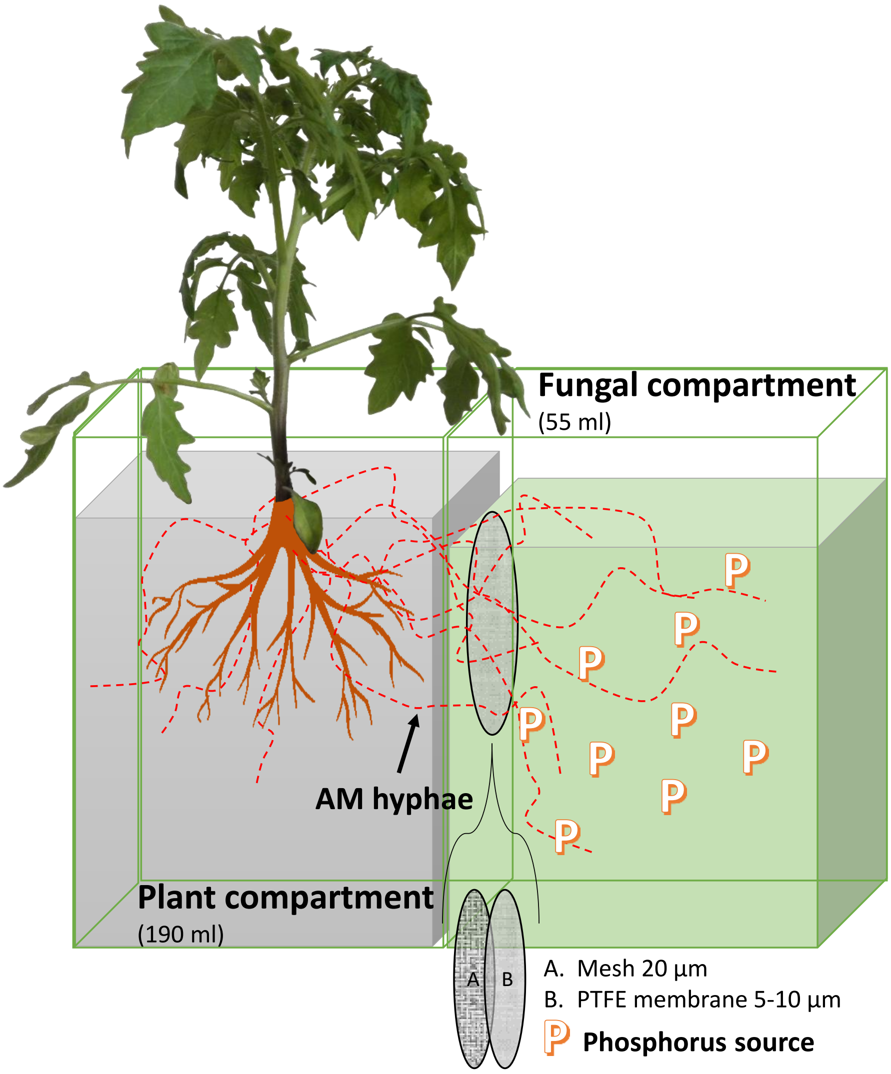
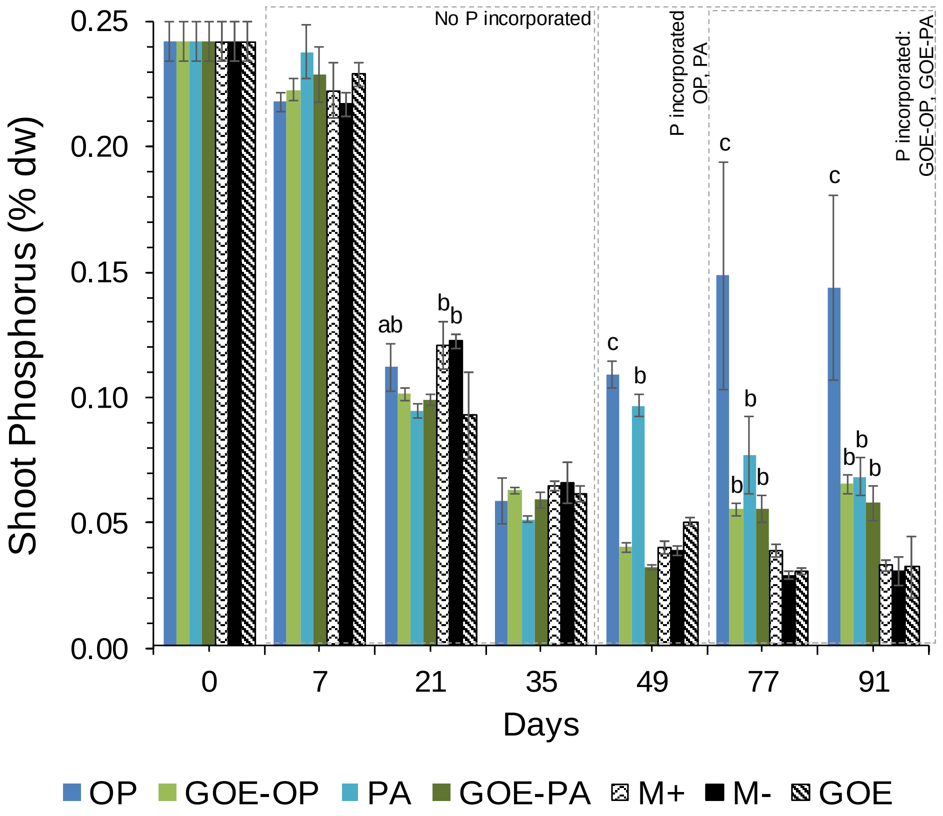


Figure 1. Scheme of the used mesocosms, comprising a plant and a fungal compartment, respectively. A polyamide mesh (20 µm pore diameter) separated mycorrhizal roots and mycelium, while the polytetrafluoroethylene (PTFE) membrane (5-10 µm pore diameter) allowed the AMF to cross and access the P sources but avoided the diffusion of ions into the plant compartment.

A


Shoot Phosphorus (% dw)
0.25
0.20
0.15
0.10
0.05
0.00
No P incorporated
P incorporated OP, PA
P incorporated: GOE-OP, GOE-PA
ab
b
b
c
b
c
b
b
b
c
b
b
b
b
0
7
21
35
49
77
91
Days
▇ OP ▇ GOE-OP ▇ PA ▇ GOE-PA ▨ M+ ■ M- ▧ GOE

B

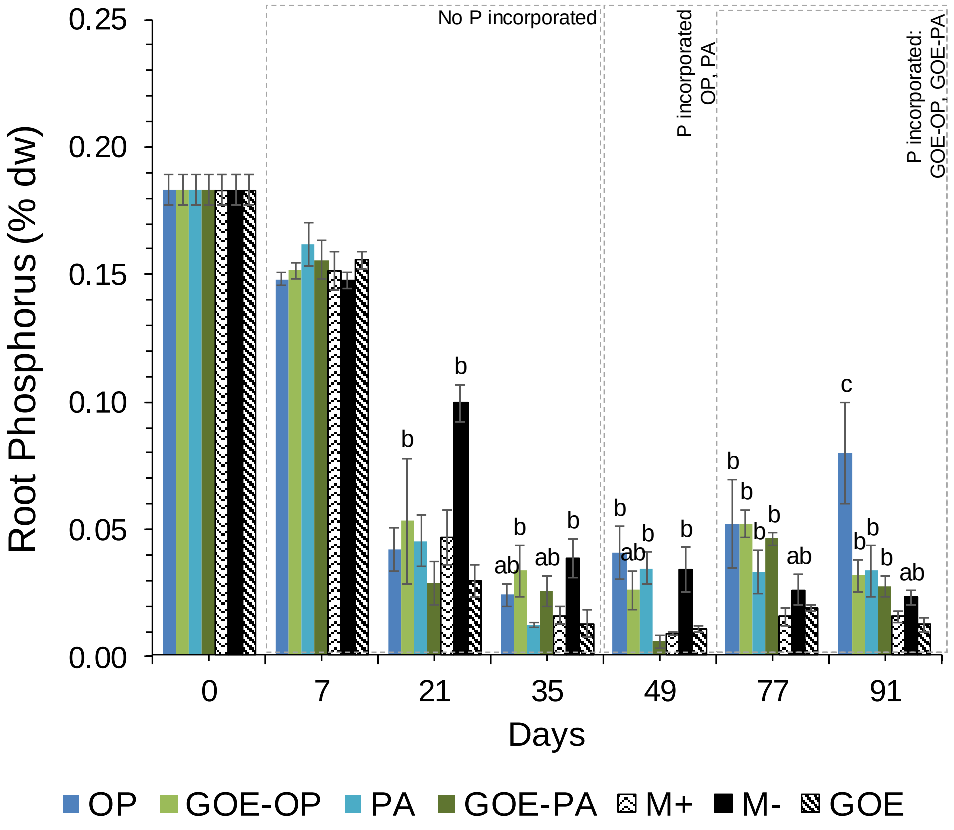

Root Phosphorus (% dw)
0.25
0.20
0.15
0.10
0.05
0.00
No P incorporated
P incorporated OP, PA
P incorporated: GOE-OP, GOE-PA
b
b
ab
b
ab
b
b
ab
b
b
b
b
b
b
ab
c
b
b
b
ab
0
7
21
35
49
77
91
Days
▇ OP ▇ GOE-OP ▇ PA ▇ GOE-PA ▨ M+ ■ M- ▧ GOE

Figure 2. Mean values and standard errors for shoot (A) and root (B) P concentration (% dry weight) in the tomato plant for the different available P sources and controls during the time course experiment. Three explicative boxes are included to differentiate the periods when the AM plants incorporated P from their respective sources. Within each P source and day, treatments with significant differences are labelled with different letters ($p < 0.05$) as result of a one-way ANOVA.

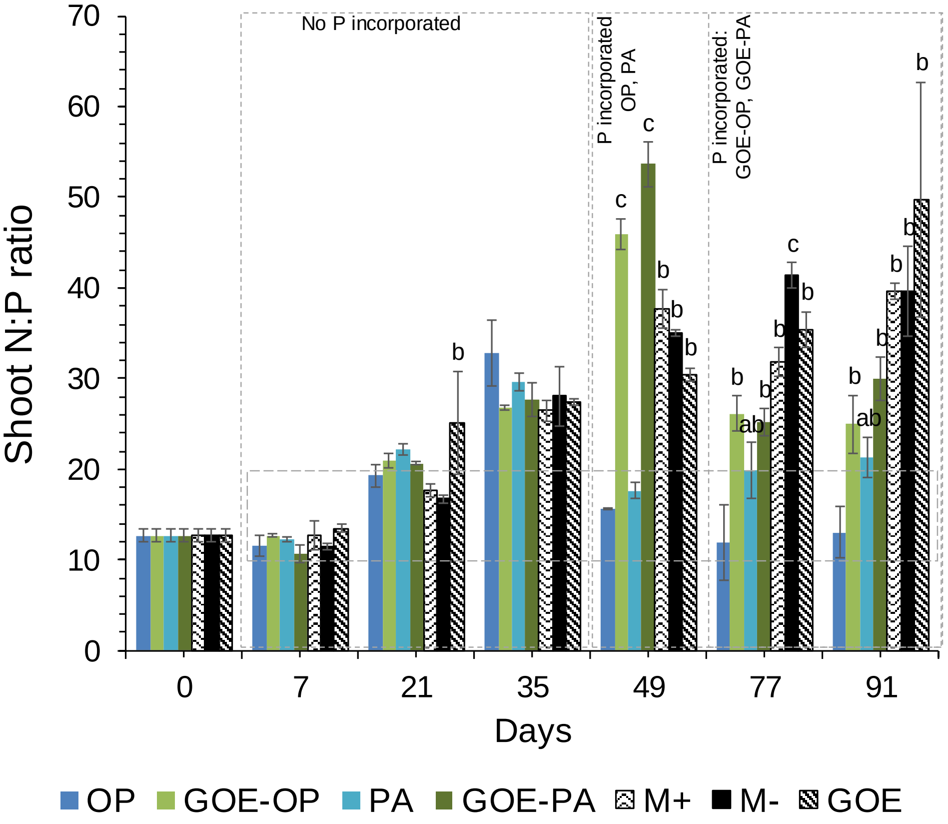


Figure 3. Mean values and standard errors for shoot N:P ratio in the tomato plant, for the different available P sources and controls during the time course experiment. A box has been drawn covering the values 10-20 of the N:P ratio, to represent the range of the ratio where no nitrogen (<10) or phosphorus (>20) deficiency would exist. Three explicative boxes are included to differentiate the periods when the AM plants incorporated P from their respective sources. Within each P source and day, treatments with significant differences are labelled with different letters ($p < 0.05$) as result of a one-way ANOVA.

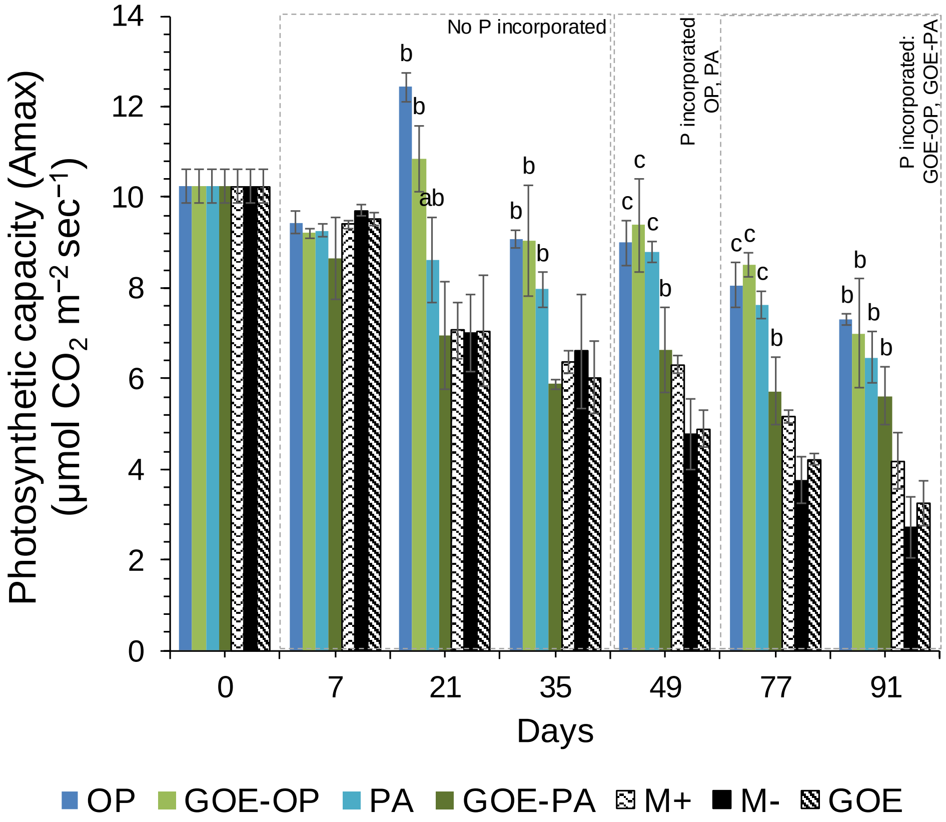
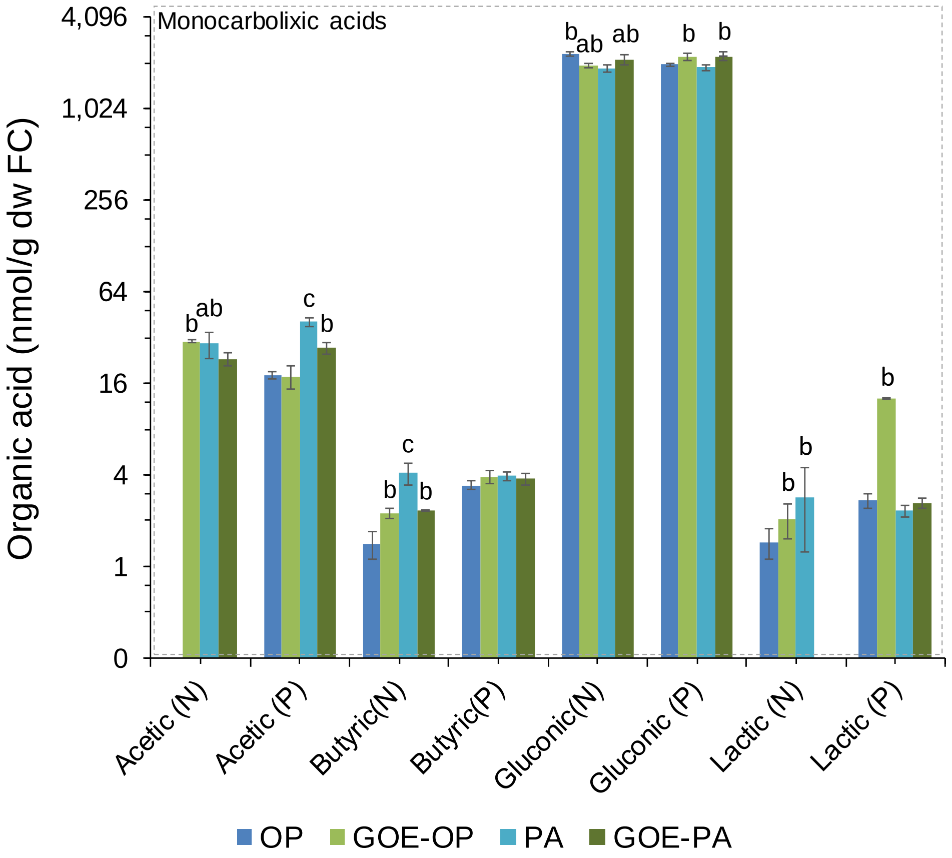


Figure 4. Mean values and standard errors for photosynthetic capacity (Amax) in tomato leaves for the different available P sources and controls during the time course experiment. Three explicative boxes are included to differentiate the periods when the AM plants incorporated P from their respective sources. Within each P source and day, treatments with significant differences are labelled with different letters ($p < 0.05$) as result of a one-way ANOVA.

A


Monocarbolixic acids
Organic acid (nmol/g dw FC)
4,096
1,024
256
64
16
4
1
0
b
ab
c
b
c
b
b
b
ab
ab
b
b
b
b
b
Acetic (N)
Acetic (P)
Butyric(N)
Butyric(P)
Gluconic(N)
Gluconic (P)
Lactic (N)
Lactic (P)
OP
GOE-OP
PA
GOE-PA

B

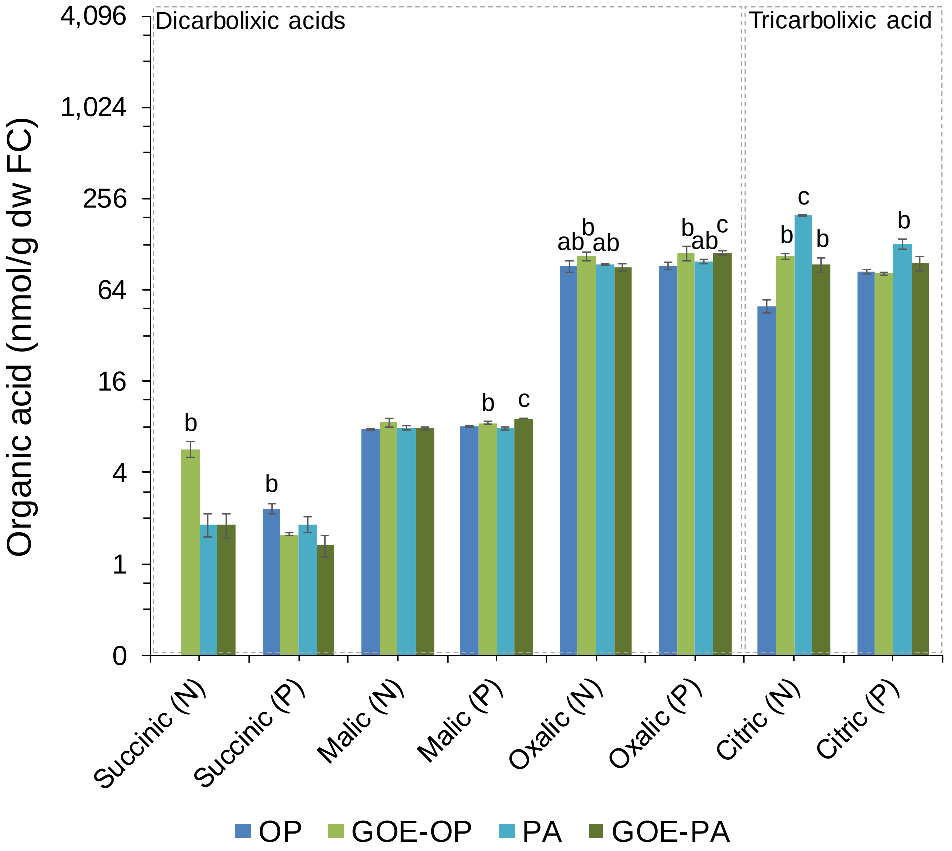
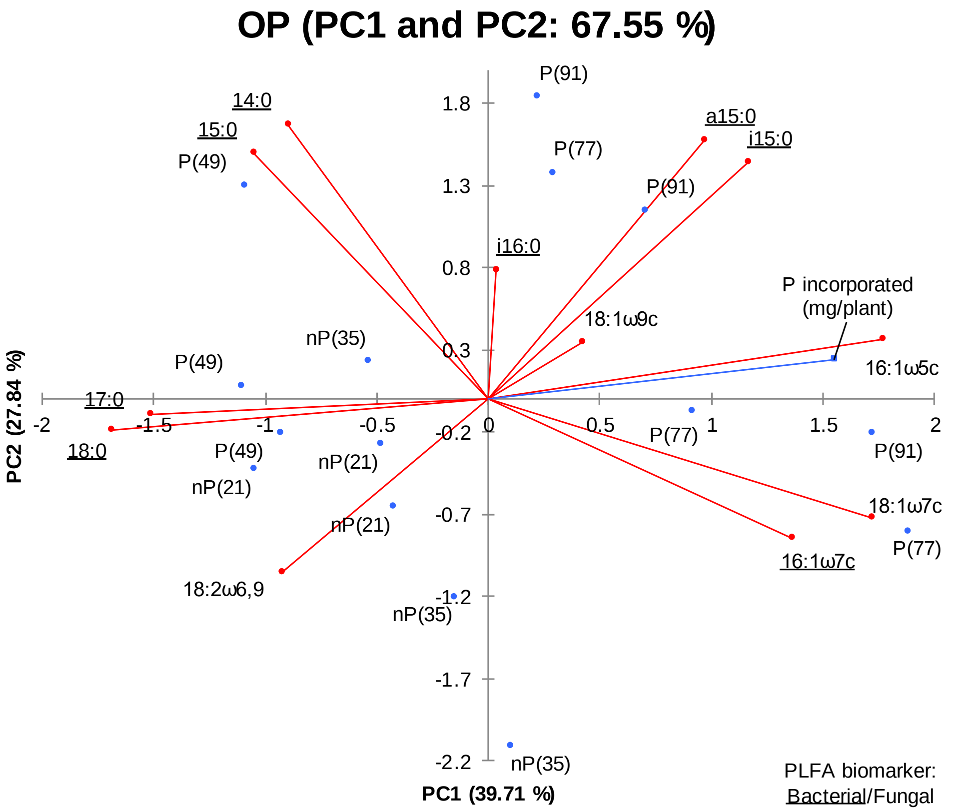
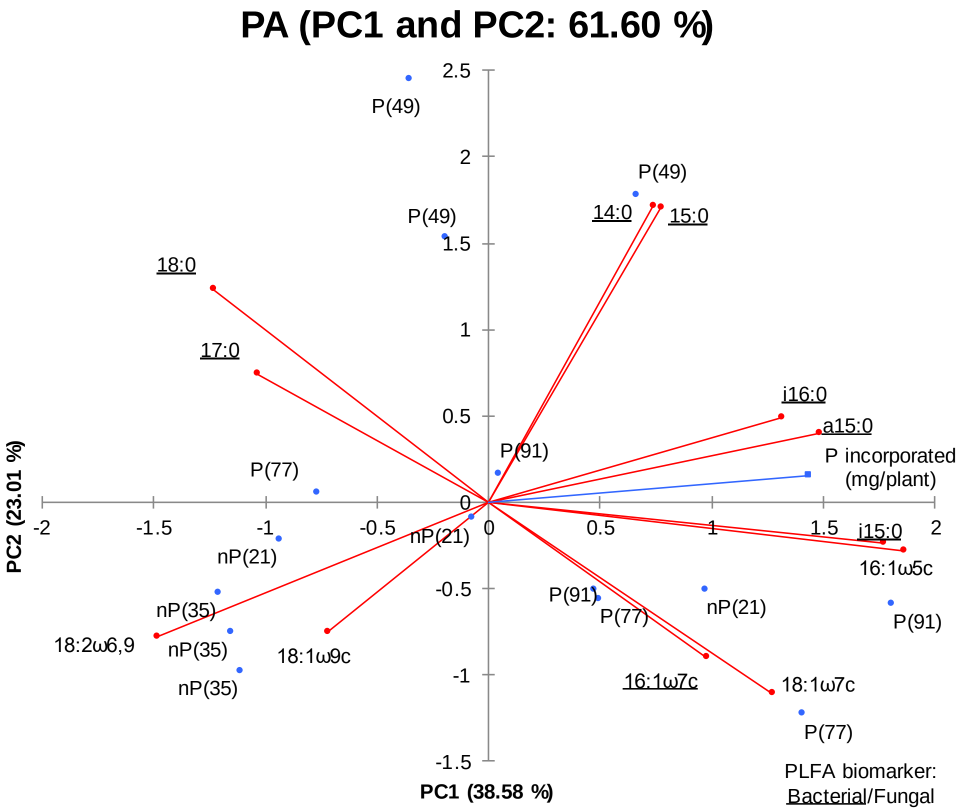

Dicarbolixic acids
Tricarbolixic acid
Organic acid (nmol/g dw FC)
4,096
1,024
256
64
16
4
1
0
b
b
b
c
ab
b
ab
b
ab
c
b
c
b
b
Succinic (N)
Succinic (P)
Malic (N)
Malic (P)
Oxalic (N)
Oxalic (P)
Citric (N)
Citric (P)
OP
GOE-OP
PA
GOE-PA

Figure 5. Low-molecular-weight organic acids (LMWOA) determined in the fungal compartment containing the different available P sources during the time course experiment. LMWOA are grouped depending on the amount of carboxylic groups (-COOH) present in their molecular structure, differentiating between mono/di/tricarboxylic acids. Based on the results from Figure 2, each bar shows the mean value and standard errors for all those samples which belong either to a period of no P incorporation (N) or to those ones with P incorporation (P), for their respective P sources. Within each test, treatments with significant differences are labelled with different letters ($P<0.05$) as result of a one-way ANOVA.

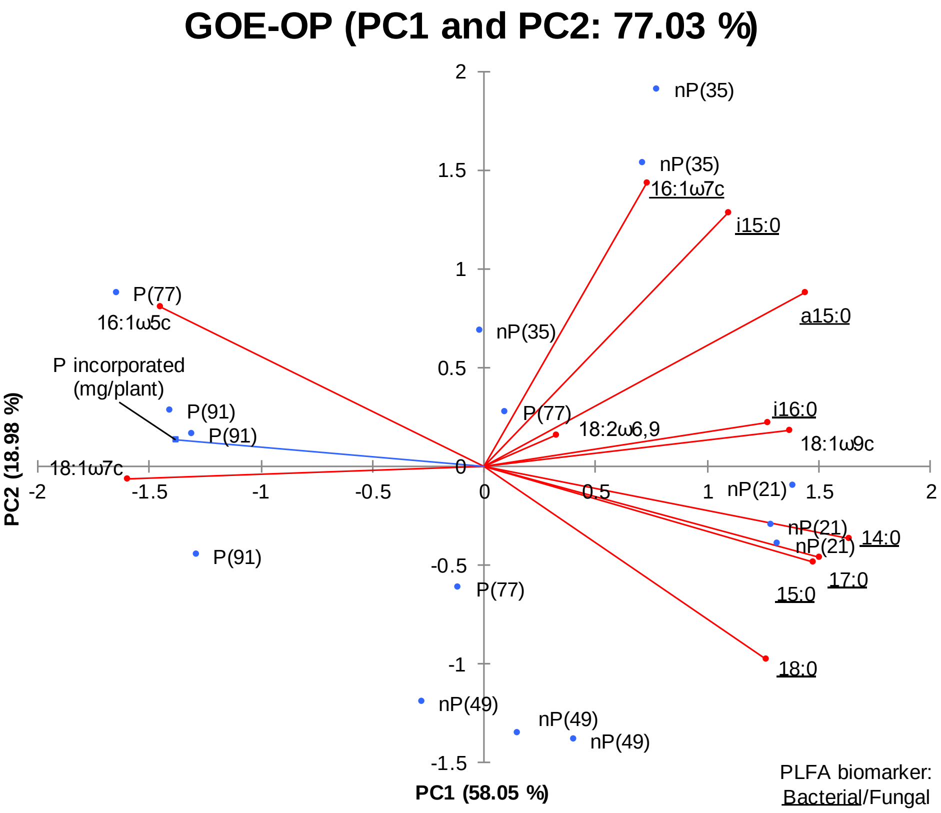
A


OP (PC1 and PC2: 67.55 %)
PC2 (27.84 %)
PC1 (39.71 %)
1.8
1.3
0.8
0.3
-0.2
-0.7
-1.2
-1.7
-2.2
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
14:0
15:0
P(49)
P(91)
P(77)
a15:0
i15:0
P(91)
i16:0
P incorporated (mg/plant)
18:1ω9c
16:1ω5c
nP(35)
P(49)
17:0
18:0
P(49)
nP(21)
nP(21)
P(77)
P(91)
nP(21)
18:1ω7c
P(77)
16:1ω7c
18:2ω6,9
nP(35)
nP(35)
PLFA biomarker:
Bacterial/Fungal

B


PA (PC1 and PC2: 61.60 %)
PC2 (23.01 %)
PC1 (38.58 %)
2.5
2
1.5
1
0.5
0
-0.5
-1
-1.5
-2
-1.5
-1
-0.5
0
0.5
1
1.5
2
P(49)
P(49)
14:0
15:0
P(49)
18:0
17:0
i16:0
a15:0
P incorporated (mg/plant)
P(91)
P(77)
nP(21)
nP(21)
i15:0
16:1ω5c
nP(35)
18:2ω6,9
nP(35)
nP(35)
18:1ω9c
P(91)
P(77)
nP(21)
P(91)
16:1ω7c
18:1ω7c
P(77)
PLFA biomarker:
Bacterial/Fungal

C


GOE-OP (PC1 and PC2: 77.03 %)

D

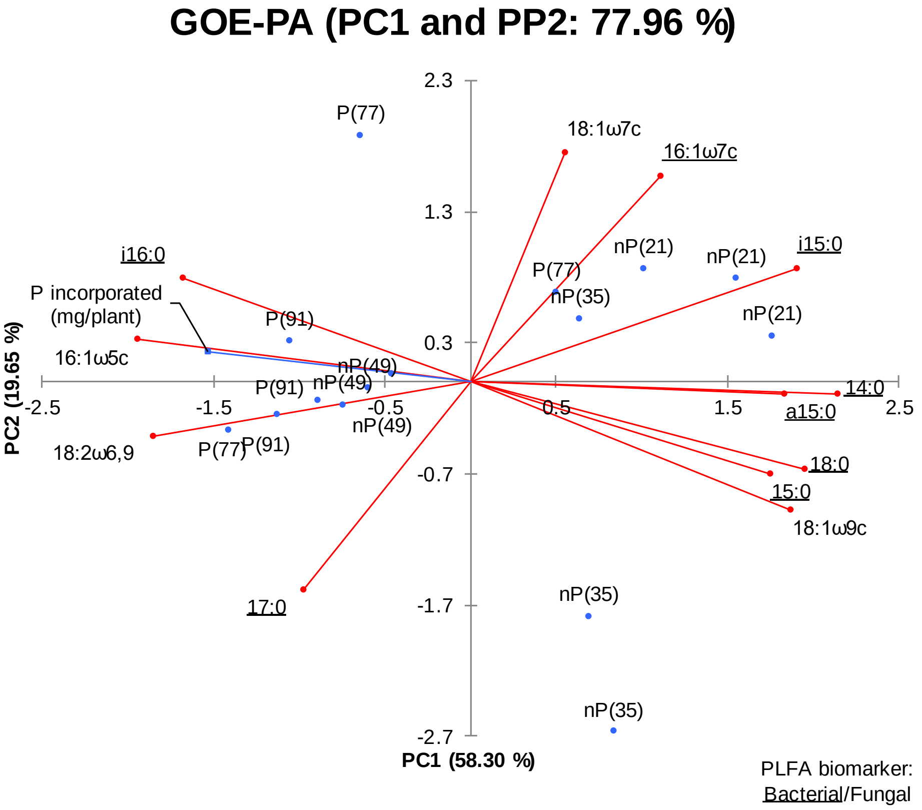

GOE-PA (PC1 and PP2: 77.96 %)

Figure 6. Two-dimensional biplots derived from principal component analysis (PCA) of PLFA profiles for the different P sources measured in the fungal compartment for each P source (A, B, C, and D). Vectors containing an underlined etiquette represent PLFA bacterial biomarkers, those ones without belong to fungal biomarkers (Table 1). Each dot represents a biological replicate (n=3) for a certain time point, the harvesting day is given in brackets. Those dots marked with a capital "P" belong to a period when AM tomato plants incorporated P from the different sources and "nP" dots to a period without P incorporation from any source. Angles between vectors indicate relationships between variables, acute ones show positive correlations, angles >90° show negative correlations between the variables. The amount of P incorporated from the different sources (mg/plant) is present as a supplementary variable named "P incorporated". The principal components were computed using only PLFA profiles, the gained P supplementary variable was projected later onto the vector subspace generated by the factors; therefore, the supplementary variable had no influence on the calculation of the PCA.

5. General discussion

Plant productivity of natural and managed ecosystems is often limited by P availability, for this reason plants evolved diverse adaptations to acquire the phosphate from the different soil P pools (Ceulemans et al., 2017; Ryan et al., 2012). In the current dissertation, I investigated the adaptation established by more than 80% of terrestrial plants with AMF, to mobilise phosphate derived from the different soil P fractions (Smith and Read, 2008). Although the AM symbiosis represents an evolutionary success in terms of soil prospection costs in search of P (Hodge, 2016), it has not been evidenced the existence of a C for P trading, depending on the P form accessibility. For this reason, in Studies 1 (S1) and 2 (S2), I concentrated the efforts on determining whether AMF can mobilise P derived from five sources with different potential accessibilities, while determining the acquisition costs imposed by the mobilisation of each of them, in terms of photoassimilate investments. Additionally, in the Study 3 (S3) I attempted to elucidate the underlying mechanisms behind the mobilisation of four of the P forms. The S1 consisted of two experiments separated temporarily. In a first test, I aimed at finding the dynamics of incorporation of P from OP, AP and PA. In the second experiment described in S1, I carried out an evaluation of the recently assimilated C that was being invested in the acquisition of each P source. The S2 and S3 are the result of a single experiment. The objectives for S2 are the same as those established in S1, while for S3, I investigated the mechanisms involved in the mobilisation of P resulting from OP, PA, GOE-OP and GOE-PA. I have structured the present discussion in different sub-sections to jointly examine the findings obtained in the three different studies, for each P form.

5.1 Orthophosphate (OP)

I tested the OP as P source in S1, 2 and 3.

All the AM plants mobilising P derived from OP incorporated the largest quantities in the shortest period of time (Figures S1.2 and S2.2), since the AMF can acquire phosphate ions in the form of OP in the soil solution (Hodge, 2016; Smith et al., 2003). *R. irregularis* not only mobilised P resulting from OP, but also stored it into the hyphae. The P content in the AM hyphae was the largest (Figure S2.4). The P stock in the fungal biomass for OP was the lowest, as the amount of mycelium of *R. irregularis* was the lowest when compared with the rest of P forms (Figure S2.3). This result agrees with findings made by Ezawa et al (2002), who found the P content in the hyphae responds flexibly to the P availability in the medium, and thus varies its internal content proportionally.

Mobilisation of P derived from OP into the AM plant was preferentially incorporated in the photosynthetic tissues (Figure S3.2). This fact was also reflected in the shoot N:P ratio, which pointed to a no P deficiency status (<20) (Figure S3.3). The photosynthetic capacity was significantly higher where N:P ratios were around 20 and below (Figure S3.4). Additionally, AM plants with access to OP exhibited significantly larger leaf areas than AM plants mobilising P derived from the PA, AP (Figure S1.9). In this regard, Birhane et al., (2012) and Parádi et al., (2003) attributed the larger leaf areas and bigger total P contents in plant tissues as the main causes for showing an enhanced photosynthetic function. Thus, the results showed that AM plants mobilising P derived from OP exhibited an enhanced photosynthetic capacity, which in turn is regulated by the AMF C sink capacity (Rich et al., 2017). The strength of the new C sink accelerates the utilization of triose phosphate for sucrose synthesis and the export towards the phloem, thus increasing plant P recycling rates when releasing P back to the chloroplast and activating the regeneration of Ribulose 1,5-bisphosphate in the Calvin Cycle. Therefore, more C is fixed per time and per unit of P, resulting in higher photosynthetic P use efficiency (Kaschuk et al., 2009; Tuomi et al., 2001). Thus, AM plants mobilising P resulting from the OP influenced positively the photosynthetic function via an enhanced photosynthetic P use efficiency, to keep a constant transfer of photoassimilates to the AMF in the fungal compartment.

I determined the respired CO_2 (mg) and the total organic C (mg) measured at the fungal compartment as proxies for the metabolic activity and the total C brought by the AMF in the fungal compartment, respectively. I observed both parameters were always significantly lower, in comparison to the other P sources (Figures S1.3, S2.5 and S2.6). Furthermore, the autotrophic CO_2 respiration and recently assimilated organic C at day 84 (Figure S1.8) exhibited the same trend. As AM plants mobilising OP exhibited significantly the lowest respired $^{13}CO_2$ (Figures S1.8 and S1.9) and ^{13}C labelled organic C (Figure S1.9), in comparison to the rest of P sources. Thus, the results showed that *R. irregularis* received significantly lower amounts of plant assimilates for mobilising OP at the fungal compartment *versus* the rest of P sources.

Moreover, I traced the fate of plant photosynthates into seventeen different phospholipid fatty acids (PLFA) (Table S1.1) and the AMF neutral lipid fatty acids 16:1ω5c (NLFA), to assess the microbial community structure (Frostegård et al., 1991; Zelles, 1999), in the fungal compartments containing the different P sources. The AM plants that incorporated P derived from OP always exhibited the lowest AMF PLFA 16:1ω5c content (extraradical mycelium) and AMF NLFA 16:1ω5c content (energy storage) (Aarle and Olsson, 2003; Bååth, 2003), per total P incorporated into the plant tissues (Figures S1.4, S1.5, S1.10, S1.12 and S2.7). The current results suggest the existence of a specific strategy to mobilise the P resulting from OP, through lower C investments in hyphae (PLFA 16:1ω5c) and storage energy (NLFA 16:1ω5c), since the OP is readily available to be mobilised by the AMF inside the hyphae (Hodge, 2016). Thus, the results support Jansa's (2011) hypothesis, whereby scavenging the readily soil P fraction would require short lived hyphae, as here a quick exploration of larger soil volumes is key and the process of scavenging OP from soil solution itself does not require larger amounts of energy, in contrast to other less accessible sources.

Mycorrhization results provided a better insight into the symbiotic interaction during the mobilisation of P derived from OP. The mycorrhizal root traits in the AM plant exhibited the occurrence of an Arum-type morphology, characterized by highly branched hyphal termini arbuscules on intercellular hyphae (Saito and Ezawa, 2016). For all AM plants mobilising P resulting from OP, the presence of arbuscules (%) correlated positively with P incorporated into the plant and the AMF PLFA biomarkers 16:1ω5c and 18:1ω7c (Table S3.4). Thus, the results indicated a clear nexus among the arbuscules, main symbiotic interface for the exchange of nutrients between the fungus and the plant (Parniske, 2008), the P incorporation into the plant tissues and the fate of photoassimilates into the AMF development in the fungal compartment.

5.2 Phytic acid (PA)

I tested the PA as P source in S1, 2 and 3.

AM plants mobilising P derived from PA incorporated similar amounts as AM plants accessing OP (Figures S1.2 and S2.2). Similar to my finding, Feng et al. (2003) observed that organic P may contribute in a comparable way as OP to the AM plant nutrition. To be available for the plant, PA must be hydrolyzed by a phosphatase (E.g. phytase, acid phosphatase) (Javaid, 2009). I did not determine enzymatic activities, but it is widely confirmed the ability of *R. irregularis* DAOM 197198 to produce acid phosphatases, and thus contribute to the mineralization of P-bearing organic compounds (Joner et al., 2000; Koide and Kabir, 2000; Sato et al., 2015; Tarafdar and Marschner, 1994; Tisserant et al., 2012). The P stock (Figure S2.3) and content (Figure S2.4) in the AM hyphae of *R. irregularis* mobilising P from PA were not significantly different to AM plants accessing OP in the fungal compartment, thus P content in hyphae responded flexibly to the P availability in the medium, varying its internal content proportionally, as predicted by Ezawa et al (2002).

Similar to AM plants accessing OP, the AM plants mobilising P derived from PA preferentially incorporated the P in the shoots *versus* the roots (Figure S3.2), showing a shoot N:P ratio below 20 (no P deficiency), once the P was incorporated into the photosynthetic tissues (Figure S3.3). Additionally, AM plants with access to PA exhibited significantly smaller leaf areas than AM plants mobilising P resulting from OP (Figure S1.9). AM plants mobilising P derived from PA did not significantly differ to OP when comparing the plant photosynthetic capacity (Figures S1.9 and S3.4). Thus, although the foliar areas were smaller than AM plants with access to OP, the photosynthetic capacity remained unaltered most likely for keeping a constant transfer of photoassimilates to the AMF.

The photosynthates invested in the AMF mobilising PA, as the sum of respired CO_2 (mg) and the total organic C (mg) measured in the fungal compartment was significantly higher, in comparison to AM plants accessing the OP (Figure S2.6). Furthermore, the respired $^{13}CO_2$ respiration and ^{13}C labelled organic C at day 84 were also the highest for AM plants mobilising PA (Figures S1.8 and S1.9). A possible consequence to mobilise P from the PA is an increased fungal metabolism, as the fungus utilizes the plant photosynthates, for its catabolic and anabolic metabolism (Jansa et al., 2011). As already noted by Nottingham et al.(2010) and Tomè et al. (2016), an increased respired CO_2 along with the total organic C content may be a normal response from the AMF to an environment where nutrients are scarce or not directly available. Thus, contrary to AM plants mobilising P from OP, the results showed that *R. irregularis* received significantly higher amounts of plant assimilates for mobilising PA at the fungal compartment.

When tracking down the course of plant photosynthates into the PLFA and NLFA fungal biomarkers, the AM plants that incorporated P derived from PA exhibited a higher production of extraradical mycelium (PLFA 16:1ω5c) and energy storage (NLFA 16:1ω5c), compared to AM plants with access to OP (Figures S1.4, S1.5, S1.10, S1.12 and S2.7). As Jansa et al. (2011) speculated, long-lived AM hyphae may represent an advantage when less accessible source as PA is mobilised. PA must be mineralized prior to its later mobilisation, unlike the AM plants incorporating OP, the results suggest the existence of a different strategy involving larger C investments in fungal infrastructure to mobilise similar amounts of P resulting from PA.

Similar to the plants accessing OP, the root mycorrhization rates also showed the occurrence of an Arum-type morphology for the plants mobilising P derived from PA. The arbuscules (%) correlated positively together with P incorporation into the AM plant tissues and the AMF PLFA biomarkers 16:1ω5c (Table S3.4), thus indicating a clear link to the symbiotic functioning between the arbuscules, the P incorporation into the plant tissues and the AMF development in the fungal compartment, along the S2-3 time course experiment.

Another important aspect when evaluating the activity of the AMF inside the fungal compartment, is the fact that AMF may act as a C pathway bypassing photoassimilates to other microbial communities intimately related to the hyphosphere (Kaiser et al., 2015). For AM plants accessing the PA at the fungal compartment, the results point towards AMF developing in parallel to gram-positive and gram negative bacterial groups (Figures S1.11 and Table S3.3), as I found values of recently assimilated C found in the gram-positive and negative bacteria populations were higher in comparison to the OP (Figure S1.11). The presence of such bacterial groups could be due to the fact that AM tomato plants were inoculated with a non-monoxenic culture of *R. irregularis* DOAM 197198, thus carrying the microorganisms naturally associated with its hyphosphere. One of the possible roles of both AMF and bacterial groups interacting together may be the production of LMWOA, as those mesocosms containing free PA in the fungal compartment exhibited the presence of significant amounts of the tricarboxylic citric acid before and after the P incorporation into the plant tissues (Figure S3.5). It is important to emphasize that the presence of citric acid in soil solution may have a synergistic effect on the secreted phosphatases (E.g. acid phosphatase), by changing the chemical structure or molecular size of the organic P and making it more accessible to enzymatic action (Otani and Ae, 1999). The nature of AM fungal exudates may be diverse, comprising phosphatases and LMWOA (Sato et al., 2015). Toljander et al. (2007) and Tawaraya et al.,(2006) exhibited that AMF may exudate LMWOA into the soil solution, showing that acetate, formiate, malate and citrate were part of the exudates. In case of bacterial groups, it has been investigated the joint action of AMF and mineralizing bacteria with the potential to improve P availability to the plant through synthetized phosphatases and LMWOA (Zhang et al., 2016). Although both groups of organisms could have contributed to the mineralization of PA through the production of phosphatases and LMWOA, the results reveal that most of the newly assimilated C (99.1%) remained within the AMF 27 hours after $^{13}CO_2$ labelling (Figure S1.10), still cannot be completely excluded the role of the accompanying gram-positive and negative bacteria in the PA mineralization process.

5.3 Apatite (AP)

I tested the AP as P source in S1.

All AM plants mobilising P resulting from AP incorporated similar amounts of P as in the case AM plants with access to OP. I observed the largest quantities of P incorporation at the end of the time course experiment (Figure S1.2). The AM plant mobilising P derived from AP incorporated more P than the available water-soluble fraction, pointing towards an active weathering of the primary mineral. Several studies have reported similar results where AM plants solely fed with AP were able to mobilise phosphate, unlike non mycorrhized treatments (Murdoch et al., 1967; Pel et al., 2018; Souchie et al., 2010; Taktek et al., 2017).

Although the mechanism for mobilising P of the AP was not elucidated in this dissertation, Koele et al. (2014) confirmed the existence of weathering tunnels under arbuscular associations coupled to P uptake from AP. They pointed out to the soil acidification as one likely mechanism for the AP weathering (E.g. LMWOA).

At day 84 (S1), when there were not significant differences of P incorporated from each source, the AM plants with access to AP showed similar photosynthetic capacity and leaf areas as plants mobilising P resulting from OP (Figure S1.9). As I explained for OP, the larger leaf areas and bigger total P content in AM plant accessing AP, were the major drivers for exhibiting an enhanced photosynthetic function, along with improved C sink fixation stemmed into the AMF, in comparison to the M+ control. Hence, the findings support that the amount of P mobilised from AP influenced the photosynthetic function and the leaf area sizes, to keep a steadily transfer of C into the AMF.

All C in the fungal compartment was exclusively transferred by the AMF during the mobilisation of the different P sources. The ratio of respired CO_2 (mg) to the total organic C (mg) measured at the fungal compartment (Figure S1.3) was higher during most of the time course experiment. Similar results were observed for the respired $^{13}CO_2$ and ^{13}C organic C at day 84 (Figures S1.8 and S1.9). The results point towards an enhanced AMF metabolism, for mobilising P from the AP. Contrary to AM plants mobilising P from OP, the results showed that *R. irregularis* received significantly higher amounts of plant assimilates for mobilising AP at the fungal compartment.

The AM plants that incorporated P derived from AP exhibited similar production of extraradical mycelium (PLFA 16:1ω5c) and energy storage (NLFA 16:1ω5c), compared to AM plants with access to OP (Figures S1.4, S1.5), but differing amounts of freshly assimilated C into the AMF biomarkers (Figures S1.10, S1.12). The AM plants with access to AP diversified the fate of recently assimilated C, incorporating it into the PLFA and NLFA 16:1ω5c, following the 27 hours after the $^{13}CO_2$ labelling. The results indicate the more stable and therefore less accessible P sources as AP received greater amounts of recent assimilated photosynthates that were invested in further growth (PLFA 16:1ω5c) and energy accumulation (NLFA 16:1ω5c) along with a potential contribution of gram negative bacteria (Figure S1.11) in the mobilisation of phosphate from both P sources. Thus, mobilisation of P deriving from AP would require long-lived AM hyphae with higher metabolic activities, in comparison to AM plants with access to OP (Jansa et al., 2011). This strategy may represent an advantage in a hardly accessible P sources like AP, since the primary mineral must be solubilized prior to its mobilisation, unlike the AM plants incorporating OP. Hence, the results suggest the existence of a different strategy involving larger C investments in fungal infrastructure and metabolism to mobilise similar amounts of P resulting from AP.

5.4 Orthophosphate and Phytic Acid Bound to Goethite (GOE-OP and GOE-PA)

I tested GOE-OP and GOE-PA as potential P sources for the *R. irregularis* in S2 and S3. I have made the discussion on both sources of phosphorus together, as they showed similar strategies when mobilising the P bound to the goethite.

All the AM plants mobilising P derived from OP or PA bound to goethite incorporated the P later and in a lesser extent, that AM plants with access to OP and PA free forms (Figures S1.2 and S2.2). It is likely that the phosphorus sources bound to goethite were assimilated more slowly because they must be desorbed from the goethite (E.g. proton extrusion and/or LMWOA release) (Owen et al., 2015). Additionally, in case of GOE-PA it was needed a further step of hydrolysis mediated by phosphatases (E.g. acid phosphatase) (Sato et al., 2015).

AMF not only supplied P derived from the GOE-OP and GOE-PA to the host plant, but also stored it into the hyphae. In contrast to AMF mobilising P from free OP and PA, the P mobilised from goethite-bound forms was uniformly distributed between the fungus and the plant (Figure S3.3). As observed by Ezawa et al (2002), the results show smaller P contents inside the hyphae growing in the fungal compartments for the less accessible sources. Furthermore, *R. irregularis* did not transfer all of the mobilised P from the goethite sources to the plant and stored it within its hyphae, may be due to the fungus strategy to store P under deficient conditions to keep a well-balanced homeostasis (Solaiman et al., 1999).

As for OP and PA, the mobilised P from the GOE-OP and GOE-PA was incorporated preferentially in the photosynthetic tissues (Figure S3.2). AM plants mobilising GOE-OP and GOE-PA did not restore initial P content at shoot level, and exhibited a slight P deficiency (>20) when comparing the N:P ratio to plants with access to OP or PA. Although there was slightly P deficiency compared, the photosynthetic capacity remained unaltered, most likely to keep a constant transfer of photoassimilates to the AMF. As I already discussed OP and PA, AM plants mobilising P bound to goethite showed an enhanced photosynthetic function most likely through an improved photosynthetic P use efficiency, in order to keep a constant transfer of photoassimilates to the AMF in the fungal compartment.

The photoassimilates invested in the AMF during the mobilisation of the P sources bound to the goethite ended up in the fungal compartment in form of organic C and respired

CO_2. Mycorrhizal plants with access to GOE-OP and GOE-PA showed larger organic C contents and respired CO_2, compared to fungal compartments containing OP, PA and no P control (M+) (Figures S2.5 and S2.6). AMF tends to develop more readily under low nutrient availability (Bryla and Eissenstat, 2005; Olsson et al., 2010), as would be the case for those AMF growing in the fungal compartments containing GOE-OP and GOE-PA. Hence, the results suggests that organic C and respired CO_2 increased more in those fungal compartments containing P bound to goethite, that the ones with free OP or PA, to mobilise the less accessible P forms.

Furthermore, I traced the fate of plant photosynthates into the AMF PLFA biomarkers (extraradical mycelium) and AMF NLFA 16:1ω5c content (energy storage) (Aarle and Olsson, 2003; Bååth, 2003), in the fungal compartments containing the GOE-OP and GOE-PA. There were significant larger amounts of PLFA 16:1ω5c and NLFA 16:1ω5c into the fungal compartments containing GOE-OP and GOE-PA compared to OP and PA (Figure S2.7). The results indicate a different growth strategy to mobilise P from the goethite-bound compounds compared to the free P forms. The fungal compartments where AM plants accessed the P forms bound to goethite showed two and three orders of magnitude more PLFA and NLFA, respectively, compared to OP and PA. Thus, the results confirm a larger C investment in energy storage and mycelium for the AMF mobilising P from the GOE-OP and GOE-PA. Hence, the mobilisation of less accessible P sources may prompt the development of an extensive and long-lasting fungal infrastructure to establish sufficient extraradical hyphae for mobilising P more efficiently to the AM plant (Jansa et al., 2011; Smith et al., 2003).

I monitored the mycorrhization rates to get information on the functioning of arbuscular mycorrhiza during the mobilisation and incorporation of P derived from GOE-OP and GOE-PA. I observed the abundance of Arum-type arbuscules (%) correlated positively with P incorporation into the plant and the AMF PLFA biomarker 16:1ω5c for both goethite P sources. This positive correlation indicates that P acquisition was linked to the development of the arbuscules in the root, the symbiotic interfaces, and the development of the AMF in the fungal compartment. Moreover, the AMF PLFA 18:1ω7c also correlated with the abundance of arbuscules (%) and with the amount of P incorporation from GOE-OP, while the AMF PLFA 18:2ω6,9 correlated with arbuscules% and P incorporation from GOE-PA (Table S3.4). The presence of the two unsaturated 18C PLFA fungal biomarkers may be indicative of a modification in the unsaturation level of the lipid membrane of *R. irregularis*, as a response to the differing accessibility of the P sources bound to goethite. A more unsaturated lipid membrane could increase its fluidity to keep its integrity compatible with an optimal membrane functionality (Calonne et al., 2010). Moreover, I also observed positive correlations between arbuscules (%), vesicles (%), total mycorrhization intensity (%)and the AMF NLFA biomarkers (energy storage) 16:1ω5c, 18:1ω7c for GOE-PA and 16:1ω5c, 18:1ω7c, 18:2ω6,9 for the GOE-OP treatment. The results point towards an accumulation of neutral lipids in the hyphae, as a reservoir for providing sufficient the energy to incorporate P from both forms bound to goethite.

Only the AM plants accessing the GOE-PA exhibited the presence of gram positive bacteria (Table S3.3) developing in parallel to *R. irregularis.* Selvakumar et al. (2016) and Lecomte et al. (2011) also described the presence of gram-positive bacteria closely associated with spore walls and mycelium of *R. irregularis.* Aditionally, Battini et al. (2016) isolated microbiota from *Rhizophagus intraradices* mycelium and found plant-growth promoting activities such as phytate mineralization, siderophore production, mineral P solubilisation, and indol acetic acid production in several representatives of gram-positive. Thus, the results may indicate a synergistic interaction between AMF and gram-positive bacteria with abilities to mineralize PA after desorption from goethite, as fungal and bacterial PLFA biomarkers increased in parallel to the P incorporation into the plant tissues. In case of GOE-PA, one of the possible roles of both AMF and gram positive bacteria interacting may be the release of LMWOA. I detected the presence of significantly higher concentrations of LMWOA in the fungal compartments containing GOE-OP and GOE-PA before and after any P was incorporated into the plant tissues, compared to the free forms and controls (Figure S3.5). I found two dicarboxylic acids, malic and oxalic acid during plant P incorporation and citric acid, before any P was incorporated in the fungal compartments containing GOE-OP and GOE-PA. The presence of the dicarboxylic and tricarboxylic LMWOA may indicate a mechanism used by *R. irregularis* with or without the aid of the associated gram-positive bacteria to desorb P from the goethite. First, a process of desorption took place by means of ligand exchange through the action of LMWOA (Owen et al., 2015), such as oxalic, succinic, and citric acid, rendering PA then mineralizable through the action of acid phosphatases secreted by the AMF (Tisserant et al., 2012) or phosphatases from the hyphospheric microbiota (Taktek et al., 2015, 2017). Thus, the results suggest AMF did more than shortening the distance that P ions must diffuse to plant roots, because *R. irregularis* took up not readily available P from GOE-OP and GOE-PA, by means of the production of LMWOA, either of bacterial or fungal origin.

6. Conclusions

From this dissertation, new knowledge on the AM plant P nutrition has been acquired. This is the first study that has systematically investigated the mobilisation of P derived from sources with differing accessibility by an AMF. The forms of P tested in this study are the most representative for their abundance in soils, namely; inorganic (OP), organic (PA), primary mineral (AP) and P forms bound to iron secondary minerals (GOE-OP and GOE-PA). Also, this study has shown for the first time a comparison of the AM plant acquisition costs to mobilise P from each of the sources. New knowledge has also been gained on the different mechanisms implemented by the plant and the fungus to acquire the different sources of P.

Although none of the hypothesis has been rejected, some of them have been modified and explained in greater detail:

H1. The AM plants took up P derived from the five P sources (OP, AP, PA, GOE-OP and GOE-PA) offered at the fungal compartment, exclusively via the mycorrhizal pathway at contrasting kinetics and accumulation rates. AM plants with access to a P source preferentially stored P in the photosynthetic tissues. The mobilised P was redistributed in different amounts into the fungus itself and the plant, which again was influenced by the P species.

H2. The mobilisation of PA and AP entailed higher investments into fungal infrastructure (PLFA) and also the highest metabolic rates (respired CO_2) compared to OP. AM plants mobilising GOE-OP and GOE-PA exhibited a greater investment of photosynthates into the production of fungal vegetative structures, energy storage (NLFA) and respired CO_2 than AM plants mobilising free OP and PA. The mobilisation of the less available P sources required longer lived mycelium infrastructure with higher metabolic rates, in contrast to the OP with a faster hyphal turnover and lower requirements of photosynthates.

H3. P mobilisation from the five P sources had a positive influence on the photosynthetic function, through an improved photosynthetic P use efficiency, in comparison to those plants without access to a P source.

H4. P incorporation in AM plants was linked to the development of the AMF together with bacteria for treatments containing AP, PA and GOE-PA, but not for GOE-OP. Additionally, the putative AMF-related PLFA biomarkers might indicate the different P sources influencing the fungal membrane lipid composition; by increasing unsaturated lipids in the case, the AMF mobilises goethite-associated P

H5. A different profile of LMWOA at each fungal compartment containing the different P sources was found. Fungal or bacterial LMWOA with two and three carboxylic groups were more abundant before and during P mobilisation from PA, GOE-PA, and GOE-OP, pointing to displacement reactions via ligand exchange as important mechanism to mobilise P.

References

Aarle, I. M. Van, and Olsson, P. A. (2003). Fungal Lipid Accumulation and Development of Mycelial Structures by Two Arbuscular Mycorrhizal Fungi Fungal Lipid Accumulation and Development of Mycelial Structures by Two Arbuscular Mycorrhizal Fungi. *Appl. Environ. Microbiol.* 69, 6762–6767. doi:10.1128/AEM.69.11.6762.

Augé, R. M., Toler, H. D., and Saxton, A. M. (2016). Mycorrhizal Stimulation of Leaf Gas Exchange in Relation to Root Colonization, Shoot Size, Leaf Phosphorus and Nitrogen: A Quantitative Analysis of the Literature Using Meta-Regression. *Front. Plant Sci.* 7, 1084. doi:10.3389/fpls.2016.01084.

Bååth, E. (2003). The use of neutral lipid fatty acids to indicate the physiological conditions of soil fungi. *Microb. Ecol.* 45, 373–383. doi:10.1007/s00248-003-2002-y.

Battini, F., Cristani, C., Giovannetti, M., and Agnolucci, M. (2016). Multifunctionality and diversity of culturable bacterial communities strictly associated with spores of the plant beneficial symbiont Rhizophagus intraradices. *Microbiol. Res.* 183, 68–79. doi:10.1016/j.micres.2015.11.012.

Birhane, E., Sterck, F. J., Fetene, M., Bongers, F., and Kuyper, T. W. (2012). Arbuscular mycorrhizal fungi enhance photosynthesis, water use efficiency, and growth of frankincense seedlings under pulsed water availability conditions. *Oecologia* 169, 895–904. doi:10.1007/s00442-012-2258-3.

Bryla, D. R., and Eissenstat, D. M. (2005). "Respiratory Costs of Mycorrhizal Associations," in *Plant Respiration* (Berlin/Heidelberg: Springer-Verlag), 207–219. doi:10.1007/1-4020-3589-6_12.

Calonne, M., Fontaine, J., Debiane, D., Laruelle, F., Grandmougin-ferjani, A., and Sahraoui, A. L. (2010). Propiconazole Toxicity on the Non-Target Organism , the Arbuscular Mycorrhizal Fungus, Glomus irregulare. *Fungicides*, 325–346.

Ceulemans, T., Bodé, S., Bollyn, J., Harpole, S., Coorevits, K., Peeters, G., et al. (2017). Phosphorus resource partitioning shapes phosphorus acquisition and plant species abundance in grasslands. *Nat. Plants* 3, 16224. doi:10.1038/nplants.2016.224.

Dalpé, Y., Trépanier, M., Sahraoui, A. L.-H., Fontaine, J., and Sancholle, M. (2012). Lipids of Mycorrhizas. *Fungal Assoc.* 9, 137–169. doi:10.1007/978-3-642-30826-0_8.

Ezawa, T., Smith, S. E., and Smith, F. A. P. (2002). P metabolism and transport in AM fungi. *Plant Soil* 244, 221–30.

Feng, G., Song, Y. . C., Li, X. . L., and Christie, P. (2003). Contribution of arbuscular mycorrhizal fungi to utilization of organic sources of phosphorus by red clover in a calcareous soil. *Appl. Soil Ecol.* 22, 139–148. doi:10.1016/S0929-1393(02)00133-6.

Frostegård, Å., Tunlid, A., and Bååth, E. (1991). Microbial biomass measured as total lipid phosphate in soils of different organic content. *J. Microbiol. Methods* 14, 151–163. doi:10.1016/0167-7012(91)90018-L.

Graham, J. H., Hodge, N. C., Morton, J. B. B., Graham, J. H., and Hodge, N. C. (1995). Fatty Acid methyl ester profiles for characterization of glomalean fungi and their These include : Fatty Acid Methyl Ester Profiles for Characterization of Glomalean Fungi and Their Endomycorrhizae †. *Appl. Environ. Microbiol.* 61, 58–64.

Hodge, A. (2016). “Accessibility of Inorganic and Organic Nutrients for Mycorrhizas,” in *Mycorrhizal Mediation of Soil: Fertility, Structure, and Carbon Storage* (Elsevier), 129–148. doi:10.1016/B978-0-12-804312-7.00008-5.

Jansa, J., Finlay, R. D., Wallander, H., Smith, F. A., and Smith, S. E. (2011). “Role of Mycorrhizal Symbioses in Phosphorus Cycling,” in *Phosphorus in Action. Biological Processes in Soil Phosphorus Cycling*, 137–168. doi:10.1007/978-3-642-15271-9.

Javaid, A. (2009). Arbuscular Mycorrhizal Mediated Nutrition in Plants. *J. Plant Nutr.* 32, 1595–1618. doi:10.1080/01904160903150875.

Joner, E. J., Van Aarle, I. M., and Vosatka, M. (2000). Phosphatase activity of extra-radical arbuscular mycorrhizal hyphae: A review. in *Plant and Soil*, 199–210. doi:10.1023/A:1026582207192.

Kaiser, C., Kilburn, M. R., Clode, P. L., Fuchslueger, L., Koranda, M., Cliff, J. B., et al. (2015). Exploring the transfer of recent plant photosynthates to soil microbes: Mycorrhizal pathway vs direct root exudation. *New Phytol.* 205, 1537–1551. doi:10.1111/nph.13138.

Koide, R. T., and Kabir, Z. (2000). Extraradical hyphae of the mycorrhizal fungus Glomus intraradices can hydrolyse inorganic phosphate. *New Phytol.* 148, 511–517. doi:10.1046/j.1469-8137.2000.00776.x.

Lecomte, J., St-Arnaud, M., and Hijri, M. (2011). Isolation and identification of soil bacteria growing at the expense of arbuscular mycorrhizal fungi. *FEMS Microbiol. Lett.* 317, 43–51. doi:10.1111/j.1574-6968.2011.02209.x.

Murdoch, C. L., Jackobs, J. A., and Gerdemann, J. W. (1967). Utilization of phosphorus sources of different availability by mycorrhizal and non-mycorrhizal maize. *Plant Soil* 27, 329–334. doi:10.1007/BF01376326.

Nottingham, A. T., Turner, B. L., Winter, K., van der Heijden, M. G. A., and Tanner, E. V. J. (2010). Arbuscular mycorrhizal mycelial respiration in a moist tropical forest. *New Phytol.* 186, 957–967. doi:10.1111/j.1469-8137.2010.03226.x.

Olsson, A., Lekberg, Y., and Hammer, E. C. (2010). mycocentric view of arbuscular mycorrhizal networks. 74, 336–345. doi:10.1111/j.1574-6941.2010.00956.x.

Olsson, P. A., van Aarle, I. M., Allaway, W. G., Ashford, A. E. A. E., and Rouhier, H. (2002). Phosphorus Effects on Metabolic Processes in Monoxenic Arbuscular Mycorrhiza Cultures. *Plant Physiol.* 130, 1162–1171. doi:10.1104/pp.009639.

Otani, T., and Ae, N. (1999). Extraction of organic phosphorus in Andosols by various methods. *Soil Sci. Plant Nutr.* 45, 151–161. doi:10.1080/00380768.1999.10409331.

Owen, D., Williams, A. P., Griffith, G. W., and Withers, P. J. A. (2015). Use of commercial bio-inoculants to increase agricultural production through improved phosphrous acquisition. *Appl. Soil Ecol.* 86, 41–54. doi:10.1016/j.apsoil.2014.09.012.

Parádi, I., Bratek, Z., and Láng, F. (2003). Influence of arbuscular mycorrhiza and phosphorus supply on polyamine content, growth and photosynthesis of Plantago lanceolata. *Biol. Plant.* 46, 563–569. doi:10.1023/A:1024819729317.

Parniske, M. (2008). Arbuscular mycorrhiza: the mother of plant root endosymbioses. *Nat. Rev. Microbiol.* 6, 763–75. doi:10.1038/nrmicro1987.

Pel, R., Dupin, S., Schat, H., Ellers, J., Kiers, E. T., and van Straalen, N. M. (2018). Growth benefits provided by different arbuscular mycorrhizal fungi to Plantago lanceolata depend on the form of available phosphorus. *Eur. J. Soil Biol.* 88, 89–96. doi:10.1016/j.ejsobi.2018.07.004.

Ryan, M. H., Tibbett, M., Edmonds-Tibbett, T., Suriyagoda, L. D. B., Lambers, H., Cawthray, G. R., et al. (2012). Carbon trading for phosphorus gain: The balance between rhizosphere carboxylates and arbuscular mycorrhizal symbiosis in plant phosphorus acquisition. *Plant, Cell Environ.* 35, 2170–2180. doi:10.1111/j.1365-3040.2012.02547.x.

Saito, K., and Ezawa, T. (2016). “Phosphorus metabolism and transport in arbuscular mycorrhizal symbiosis,” in *Molecular Mycorrhizal Symbiosis* (Hoboken, NJ, USA: John Wiley & Sons, Inc.), 197–216. doi:10.1002/9781118951446.ch12.

Sato, T., Ezawa, T., Cheng, W., and Tawaraya, K. (2015). Release of acid phosphatase from extraradical hyphae of arbuscular mycorrhizal fungus Rhizophagus clarus. *Soil Sci. Plant Nutr.* 61, 269–274. doi:10.1080/00380768.2014.993298.

Selvakumar, G., Krishnamoorthy, R., Kim, K., and Sa, T. M. (2016). Genetic diversity and association characters of bacteria isolated from arbuscular mycorrhizal fungal spore walls. *PLoS One* 11, 1–16. doi:10.1371/journal.pone.0160356.

Smith, S. E., and Read, D. J. (2008). *Mycorrhizal symbiosis*. Academic Press doi:10.1016/B978-0-12-370526-6.X5001-6.

Smith, S. E., Smith, F. A., and Jakobsen, I. (2003). Mycorrhizal fungi can dominate phosphate supply to plants irrespective of growth responses. *Plant Physiol.* 133, 16–20. doi:10.1104/pp.103.024380.

Solaiman, M. Z., Ezawa, T., Kojima, T., and Saito, M. (1999). Polyphosphates in intraradical and extraradical hyphae of an arbuscular mycorrhizal fungus, Gigaspora margarita. *Appl. Environ. Microbiol.* 65, 5604–5606.

Souchie, E. L., Azcón, R., Barea, J. M., Silva, E. M. R., and Saggin-Júnior, O. J. (2010). Enhancement of clover growth by inoculation of P-solubilizing fungi and arbuscular mycorrhizal fungi. *An. Acad. Bras. Cienc.* 82, 771–777. doi:10.1590/S0001-37652010000300023.

Taktek, S., St-Arnaud, M., Piché, Y., Fortin, J. A., and Antoun, H. (2017). Igneous phosphate rock solubilization by biofilm-forming mycorrhizobacteria and hyphobacteria associated with Rhizoglomus irregulare DAOM 197198. *Mycorrhiza* 27, 13–22. doi:10.1007/s00572-016-0726-z.

Taktek, S., Trépanier, M., Servin, P. M., St-Arnaud, M., Piché, Y., Fortin, J.-A. A., et al. (2015). Trapping of phosphate solubilizing bacteria on hyphae of the arbuscular mycorrhizal fungus Rhizophagus irregularis DAOM 197198. *Soil Biol. Biochem.* 90, 1–9. doi:10.1016/j.soilbio.2015.07.016.

Tarafdar, J. C., and Marschner, H. (1994). Phosphatase activity in the rhizosphere and hyphosphere of VA mycorrhizal wheat supplied with inorganic and organic phosphorus. *Soil Biol. Biochem.* 26, 387–395. doi:10.1016/0038-0717(94)90288-7.

Tawaraya, K., Naito, M., and Wagatsuma, T. (2006). Solubilization of insoluble inorganic phosphate by hyphal exudates of arbuscular mycorrhizal fungi. *J. Plant Nutr.* 29, 657–665. doi:10.1080/01904160600564428.

Tisserant, E., Kohler, A., Dozolme-Seddas, P., Balestrini, R., Benabdellah, K., Colard, A., et al. (2012). The transcriptome of the arbuscular mycorrhizal fungus Glomus intraradices (DAOM 197198) reveals functional tradeoffs in an obligate symbiont. *New Phytol.* 193, 755–769. doi:10.1111/j.1469-8137.2011.03948.x.

Toljander, J. F., Lindahl, B. D. B. D., Paul, L. R., Elfstrand, M., and Finlay, R. D. (2007). Influence of arbuscular mycorrhizal mycelial exudates on soil bacterial growth and community structure. *FEMS Microbiol. Ecol.* 61, 295–304. doi:10.1111/j.1574-6941.2007.00337.x.

Tomè, E., Ventura, M., Folegot, S., Zanotelli, D., Montagnani, L., Mimmo, T., et al. (2016). Mycorrhizal contribution to soil respiration in an apple orchard. *Appl. Soil Ecol.* 101, 165–

173. doi:10.1016/j.apsoil.2016.01.016.

Turner, B. L. (2008). Resource partitioning for soil phosphorus: A hypothesis. *J. Ecol.* 96, 698–702. doi:10.1111/j.1365-2745.2008.01384.x.

Zelles, L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biol. Fertil. Soils* 29, 111–129. doi:10.1007/s003740050533.

Zhang, L., Xu, M., Liu, Y., Zhang, F., Hodge, A., and Feng, G. (2016). Carbon and phosphorus exchange may enable cooperation between an arbuscular mycorrhizal fungus and a phosphate-solubilizing bacterium. *New Phytol.* 210, 1022–1032. doi:10.1111/nph.13838.

Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die bei der Naturwissenschaftliche Fakultät der Gottfried Wilhelm Leibniz Universität Hannover zur Promotionsprüfung vorgelegte Arbeit mit dem Titel:

Carbon and phosphorus trading in the arbuscular mycorrhizal symbiosis

am Lehrstuhl für Bodenkunde unter der Anleitung und Betreuung von Prof. Dr. Georg Guggenberger gemäß §6(1) der Promotionsordnung eigenständig verfasst habe. Ich versichere, dass ich keine anderen, außer den genannten Literaturquellen und Hilfsmitteln, verwendet habe. Ich habe die Dissertation in dieser oder ähnlicher Form in keinem anderen Prüfungsverfahren als Prüfungsleistung vorgelegt.

Lebenslauf

Alberto Andrino de la Fuente
andrino@ifbk.uni-hannover.de
Nationality: Spanish
Date of birth: 12th July 1981
Telephone: 017678935365

LABORAL EXPERIENCE

Position: **PhD. student**
Institution**: INSTITUT FÜR BODENKUNDE (LEIBNIZ UNIVERSITÄT HANNOVER) (http://www.soil.uni-hannover.de)**
Date: 01/10/13- nowadays
Localization: **Hannover. Germany**
Job Description

- **Plant-Soil-Interface (http://www.psi.uni-hannover.de):** my PhD project is based on the concept that storage and turnover of soil organic carbon is strongly affected by the carbon economy of mycorrhizal symbioses. Our basic hypothesis is that the performance of nutrient acquisition by the mycorrhiza controls the carbon flux belowground.
- **Process-based & Resilience-Oriented management of DIversity Generates sustainable Yields (PRODIGY)** – a transnational project in the MAP-region (Brazil, Peru, Bolivia). BMBF, Programm: Kipppunkte, Dynamik und Wechselwirkungen von sozialen und ökologischen Systemen (BioTip).

Position: **Research and Development Technician**
Company: **THADER BIOTECHNOLOGY (http://www.thaderbiotechnology.es/eng)**
Date: 19/07/10 – 31/08/13.
Localization: **Murcia. Spain**
Job Description

- ***In vitro* plant culture techniques.**
- **Elaboration of mycorrhizal plant production protocols**.
- **Technology transfer**: Argentina -Spegazzini Institute, La Plata -; United Arab Emirates - Barari Forest Management-
- Use of **GIS** (geographic information software) to elaborate **thematic cartographies**
- Use of agricultural wastes for **edible mushroom production**
- Isolation and production of **arbuscular mycorrhiza; bacteria lineage** for agricultural use; **bio-control organisms.**

Position: **Research assistant**
Company: **MURCIA UNIVERSITY (http://www.um.es)**
Date: 01/01/09 – 18/07/10
Localization: **Murcia. Spain**

Job Description

- Research contract to develop **new mycorrhizal plant production protocols**. Development of a **patent** with a new method of mycorrhizal plant production (see publications below).

Position: **Laboratory technician**
Company: **THADER BIOTECHNOLOGY (http://www.thaderbiotechnology.es/eng)**
Date: 01/10/07 - 30/12/08.
Localization: **Murcia. Spain**
Job Description

- Handling and use of **vesiculo arbuscular mycorrhizas** for forest and horticultural plants.
- ***In vitro* plant culture**: plant micropropagation, media preparation, lineage maintenance, explants selection, explant acclimation. Nursery maintenance tasks.
- Use of agricultural wastes for **edible mushroom** production.
- Production and use of **bio-control organism** *Trichoderma* spp.

Position: **Laboratory technician**
Company: **UNITED LABORATORIES (http://www.unilabs.es)**
Date: 29/07/02 - 31/10/05.
Localization: **Madrid. Spain**
Job Description

- Laboratory technician at Virgen del Mar Hospital.

EDUCATION

- Master Degree of Plant physiology and biotechnology advances at Murcia University (2009-2010).
- Licenciatura Degree Biology studies at the Autónoma de Madrid University (2002-2008).
- Laboratory technician studies at Benjamín Rua Institute. Madrid. (2000-2002).

Publications

Journals

- Boy, J., R. Godoy, O. Shibistova, D. Boy, R. McCulloch, A. Andrino, M.A. Morales, R. Mikutta, and G. Guggenberger (2016): Successional patterns along soil development gradients formed by glacier retreat in the Maritime Antarctic, King George Island., Revista Chilena de Historia Natural 89, Art. No. 6. DOI: 10.1186/s40693-016-0056-8
- Navarro-Ródenas, A., L.M. Berná, C. Lozano-Carrillo, A. Andrino, and A. Morte (2016):Beneficial Native Bacteria Improve Survival and Mycorrhization of Desert Truffle Mycorrhizal Plants in Nursery Conditions., Mycorrhiza 26 (7), 769–779. DOI: 10.1007/s00572-016-0711-6
- Andrino, A., A. Morte, M. Honrubia (2011): Characterization and culture of three *Pleurotus eryngii* (Fr). Quél strains on food and agriculture wastes., Anales de Biología. Issue 33. p. 54-67. Murcia University, Murcia, Spain.

Bücher

- Honrubia, M., A. Andrino, and A. Morte (2014): Preparation and Maintenance of Both Man-Planted and Wild Plots., In V. Kagan-Zur, N. Roth-Bejerano, Y. Sitrit, and A. Morte (Eds.), Desert Truffles (Vol. 38, pp. 367–387). Springer Berlin Heidelberg. DOI: 10.1007/978-3-642-40096-4_22
- Morte, A. and A. Andrino (2014): Domestication: Preparation of Mycorrhizal Seedlings., In V. Kagan-Zur, N. Roth-Bejerano, Y. Sitrit, and A. Morte (Eds.), Desert Truffles (Vol. 38, pp. 343–365). Springer Berlin Heidelberg. DOI: 10.1007/978-3-642-40096-4_21
- Honrubia, M., J. Fajardo, A. Andrino, A. Verde, A. Honrubia, D. Blanco, B. Bustamante, I. Martínez-Morales (2012): Micotrufa Project Guide. Vol. 1 and 2. Edible fungal species thematic cartography., MAGRAMA (Ministry of Agriculture, Food and Environment).
- Morte, A., A. Andrino, M. Honrubia, A. Navarro-Ródenas (2012): Terfezia cultivation in arid and semiarid soils., In: A. Zambonelli and G. M. Bonito (Eds.), Edible Ectomycorrhizal Mushrooms. Soil Biology, Springer-Verlag Berlin. Heidelberg.

Patent

- Andrino, A. Morte, M. Honrubia. 2011. Method for the production of mycorrhizal Cistaceae plants with desert truffle. Invention Patent. Registry number: WO2012110673A1