Regulation of phosphate and sugar transporters during the mycorrhizal symbiosis in response to phosphate availability and accessibility

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Abstract

The mycorrhizal symbiosis is formed by 70-90 % of land plants species, and its most common form is the arbuscular symbiosis (AM). It is formed between plants and soil-borne mycorrhizal fungi, which trade nutrients such as phosphate and nitrogen against plant-derived carbohydrates. AM fungi build little tree-like structures inside the plant cells, called arbuscules. Their periarbuscular membrane (PAM) is the main side of trading between the plant and the fungus. The trading process is accompanied by the upregulation of mycorrhizal transporter genes inside the plant and the fungus. On both sides, phosphate and sugar transporters have been discovered, which are regulated in response to the symbiosis. The controlling processes in the symbiosis are not entirely understood up to now. Expression of the plant phosphate transporter *PT4* has been studied, but its regulation in response to environmental factors such as different phosphate availabilities and phosphate sources is yet unknown. The regulation of the sugar transporters *SlSUT1* and *SlSUT2* from tomatoes, which have been described to be upregulated in mycorrhized plants, has not been studied so far at all. And the research about the fungal transporters *GiPT* and *GiMST2* has been focused on the extraradical mycelium, while their expression pattern in the intraradical mycelium remained unclear. Furthermore, the plant carbon investment into the fungus in response to different phosphate sources and accessibilities has not been explored in detail so far. Therefore, two experimental set-ups were developed to investigate these aspects of the symbiosis. The first was a split-root system, in which *Solanum lycopersicum* and *Medicago truncatula* plants, mycorrhized with the fungus *Rhizophagus irregularis*, could be fertilized with two different phosphate sources in different areas of their root system. One side was fertilized with either a phosphate solution or apatite as a mineral phosphate source, while the other side was not fertilized with phosphate at all. This set-up allowed us to study the gene-regulation in different root patches in response to the phosphate source available to plant and fungus. Additionally, a $^{13}CO_2$ labeling gave insight into the carbon investment of the plant. In the second experiment, only the hyphae of the mycorrhized plant had access to one of three different phosphate sources with varying accessibilities, namely apatite, phytate, and a phosphate solution. Analysis of the set-ups was done regularly over a period of 24 weeks to ensure that changes in carbon investment and gene expression over time could be detected. The C investment of the plant changed during the experiment, and differences in investment strategies according to the accessibility of the phosphate source could be observed. More carbon was invested into the hyphae to gain the same amount of P when the phosphate source was harder to access. The tomato phosphate transporter *LePT4* was downregulated significantly when no phosphate was available to the plant. Hence, the phosphate availability in the soil has a major influence on its local regulation and *LePT4* cannot be used as a marker for the degree of mycorrhization. For *M. truncatula*, there was a distinct correlation of *MtPT4* to the mycorrhization of the roots in all repetitions, but no correlation to the phosphate status of the soil could be observed, which justifies its use as a mycorrhizal marker. Apparently, the *PT4* transporters are regulated differently in different plant species. The expression of the sugar transporters *SlSUT1* and *SlSUT2* did not change in response to the phosphate availability of the soil in both experiments. However, *SlSUT1* was downregulated in mycorrhized compared to nonmycorrhized plants, in contrast to prior findings. Its role in the mycorrhizal symbiosis therefore has to be reconsidered. Fungal transporter genes were upregulated in the intraradical mycelium in response to P-starvation in both plant species, which further supports the theory of a competition over phosphate at the periarbuscular membrane.

Keywords: mycorrhizal symbiosis, phosphate transport, gene expression regulation

Zusammenfassung

Die Mykorrhizasymbiose wird von 70-90 % der Landpflanzen gebildet und ihre verbreitetste Form ist die arbuskuläre Mykorrhizierung (AM). Sie entsteht zwischen Pflanzen und bodenbürtigen Mykorrhiza Pilzen, welche Nährstoffe wie Phosphat und Nitrat gegen Kohlenhydrate der Pflanze eintauschen. AM Pilze bilden kleine, baumartige Strukturen innerhalb der Pflanzenzellen, welche Arbuskel genannt werden. Deren periarbuskuläre Membran ist die Hauptschnittstelle für den Austausch zwischen Pflanze und Pilz. Auf beiden Seiten wurden Transportergene gefunden, deren Expression durch die Symbiose erhöht ist. Die Kontrollprozesse innerhalb der Symbiose sind allerdings bisher nicht ganz erforscht. Die Expression des pflanzlichen Phosphattransporters *PT4* wurde zwar eingehend untersucht, jedoch wurde seine Regulation unter Einfluss von Faktoren wie eine unterschiedliche Phosphatverfügbarkeit im Boden oder unterschiedliche Phosphatquellen noch nicht abschließend geklärt. Die Regulationsmechanismen der Zuckertransporter *SlSUT1* und *SlSUT2* aus Tomate, deren Expression laut Literatur in mykorrhizierten Pflanzen stark erhöht ist, wurden bisher nicht weiter untersucht. Und die Forschung über die pilzlichen Transporter *GiPT* und *GiMST2* beschränkt sich größtenteils auf das extrazelluläre Mycelium. Außerdem ist die Kohlenstoffinvestition der Pflanze in den Pilz bei verschiedenen Phosphatquellen mit unterschiedlichen Verfügbarkeiten noch größtenteils unerforscht. Wir haben daher zwei Experimente entwickelt, um diese Aspekte der Symbiose genauer zu untersuchen. Das erste Experiment besteht aus einem split-root System, in dem mit dem Pilz *Rhizophagus irregularis* mykorrhizierte *Solanum lycopersicum* und *Medicago truncatula* Pflanzen mit zwei verschiedenen Phosphatquellen in unterschiedlichen Wurzelbereichen gedüngt werden konnten. Eine Seite wurde mit einer Phosphatlösung oder mit dem Mineral Apatit gedüngt, während die andere Seite keine Phosphatdüngung bekam. Dieses System hat es uns erlaubt, die Genregulation in Bezug auf verschiedene Phosphatsorten und –verfügbarkeiten gleichzeitig in verschiedenen Wurzelbereichen zu untersuchen. Zusätzlich wurde eine $^{13}CO_2$ Markierung durchgeführt, um mehr über die Kohlenstoff-Investitionen der Pflanze zu erfahren. Im zweiten Experiment hatten nur die Hyphen der mykorrhizierten Pflanzen Zugang zu drei Phosphatquellen unterschiedlicher Verfügbarkeit. Genutzt wurden hierfür Apatit, Phytate und eine Phosphatlösung. Die Analyse des Experiments geschah regelmäßig über einen Zeitraum von 24 Wochen, um Änderungen in der Kohlenstoffverteilung und der Genexpression während dieses Zeitraums verfolgen zu können. Die Kohlenstoffinvestition der Pflanze änderte sich während des Experiments, und Unterschiede in der Investitionsstrategie in Bezug auf die Zugänglichkeit der Phosphatquellen konnten beobachtet werden. Es wurde mehr Kohlenstoff von der Pflanze investiert um die gleiche Menge Phosphat zu erhalten, wenn die Quelle schwerer für den Pilz verfügbar war. Der Phosphattransporter *LePT4* der Tomate war signifikant runterreguliert, wenn gar kein Phosphat für die Pflanze zur Verfügung stand. Daher spielt die Phosphatverfügbarkeit eine wichtige Rolle in seiner lokalen Regulation und *LePT4* kann nicht als Marker für den Grad der Mykorrhizierung verwendet werden. Für *M. truncatula* konnte eine deutliche Korrelation zwischen *MtPT4* Expression und der Mykorrhizierung in allen Wiederholungen festgestellt werden, jedoch keine Korrelation zum Phosphatstatus des Bodens oder der Pflanze. Der *PT4* Transporter scheint in verschiedenen Pflanzenspezies unterschiedlich reguliert zu sein. Die Expression der Zuckertransporter *SlSUT1* und *SlSUT2* hat sich auf Grund der Phosphatverfügbarkeit in beiden Experimenten nicht verändert. Jedoch war *SlSUT1*, entgegen früherer Ergebnisse, in mykorrizierten verglichen mit nicht mykorrhizierten Pflanzen runterreguliert. Seine Rolle in der Mykorrhizasymbiose sollte darum neu überdacht werden. In beiden Pflanzenspezies waren die pilzlichen Transportergene bei akutem Phosphatmangel im intrazellulären Mycelium hochreguliert, was die Theorie eines Wettbewerbs um Phosphat an der periarbuskulären Membran weiter unterstützt.

Schlagwörter: Mykorrhiza Symbiose, Phosphattransport, Genexpressionsregulierung

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List of Abbreviations

A	Apatite
ACCase	Acetyl-CoA carboxylase
ACP	Acyl carrier protein
AM	Arbuscular mycorrhiza
AMF	Arbuscular mycorrhizal fungi
ATP	Adenosine triphosphate
EA	Elementar analyzer
EMF	Ectomycorrhizal fungi
ERM	Extraradical mycelium
GC	Gas chromatograph
HC	Hyphal compartment
IAEA	International Atomic Energy Agency
ICP-MS	Inductively coupled plasma mass spectrometry
IRM	Intraradical mycelium
IRMS	Isotopic ratio mass spectrometer
MST	Monosaccharide transporter
N	No phosphate fertilization
NLFA	Neutral lipid fatty acid
OAA	Oxaloacetic acid
P	Phosphate
PAM	Periarbuscular membrane
PCR	Polymerase chain reaction
Pi	Orthophosphate
PLFA	Phospholipid fatty acid
PPA	Prepenetration apparatus
PSB	Phytase-producing bacteria
PT	Phosphate transporter
RC	Root compartment
RNA	Ribonucleic acid
SUT	Sucrose transporte
TAG	Triacylglyceride
VPDB	Vienna Pee Dee Belemnite

1. Introduction

1.1 Mycorrhizal Symbiosis and its Characteristics

Mycorrhiza is a mutualistic symbiosis between the roots of terrestrial plants and soil-borne mycorrhizal fungi (Frank 1885). The name is derived from the Greek expressions for the words fungus (*mykos*) and roots (*rhiza*) (Frank 1885). The symbiosis is based on an exchange of water and nutrients from the fungal side for carbohydrates from the plant side.

There are two main forms of mycorrhiza, the ecto- and the endomycorrhiza. In ectomycorrhiza, the fungus does not grow inside of the plant root cells. The fungus forms a network of hyphae in the soil, the extraradical mycelium (ERM), and a mantle of fungal tissue enclosing the plant rootlet. From it, the hyphae grow into the roots between the epidermal and the cortical cells, forming a complex intercellular system called the Hartig net (Smith & Read 2008). This is the predominant form on trees in temperate forests (Parniske 2008). In endomycorrhiza, however, the fungus grows directly inside the plant root cells. The most common form of endomycorrhiza is the arbuscular mycorrhiza (AM). It is formed by 70-90 % of the land plant species including angiosperms, gymnosperms, the sporophytes of pteridophytes and the gametophytes of some hepatics and pteridophytes. It is therefore the most common terrestrial symbiosis (Smith & Read 2008). On the fungal site, it is formed by the obligatory symbiotic members of the phylum Glomeromycota (Schüßler et al. 2001). Typical for AM is the formation of characteristic structures inside the cortical cells of the plant root and also some colonized mycothalli (Smith & Read 2008). They look like little trees and are therefore called arbuscules, from the Latin word *arbusculum,* which means bush or little tree (Parniske 2008). These structures are also believed to be the main site of nutrient exchange between the plant and the fungus (Parniske 2008). The development of the symbiosis starts with the presymbiotic phase. The fungal spores germinate due to the perception of plant-derived Strigolactones and hyphae start growing and branching (Akiyama et al. 2005; Kretzschmar et al. 2012). The fungus then starts producing signaling molecules called Myc factors, which may induce symbiosis-specific responses in the plant roots. Furthermore, a hypophodium is formed by the fungus, which is a flattened, hyphal organ directly on the root cells. The plant reacts by building a prepenetration apparatus (PPA), a cytoplasmic bridge across the vacuole (Genre et al. 2005; Genre et al. 2008). Only after completion of the PPA, the fungus can penetrate the host cell and the intraradical mycelium (IRM) can grow into the apoplastic space of the cortical parenchyma and form arbuscules within the root cells (Parniske 2008; Bücking et al. 2012). The arbuscules are surrounded by a periarbuscular membrane (PAM) derived from the plant plasma membrane. The space between the PAM and the fungal plasmamembrane is called the periarbuscular space (Harrison 2005). The arbuscules grow until their

maximal size is reached and then start degradation, in which the arbuscular hyphae is separated from the remaining cytoplasm by septation (Javot, Penmetsa, et al. 2007). The arbuscular lifecycle lasts for approximately 8.5 days and is therefore shorter than that of a plant cell (Alexander et al. 1989). In later stages of the fungal development, vesicles are formed inside or between the cortical cells as storage organs. At the same time, the ERM is further developed outside of the plant roots. This hyphal network increases the exploration zone in the soil available for nutrient uptake and makes it possible for the fungus to invade more host plants (Smith & Read 2008). To complete the life cycle, spores are built by the ERM in the soil (Parniske 2008; Bücking et al. 2012). Figure 1.1 gives an overview of the steps in arbuscular development.

Both partners in the symbiosis profit from the interaction. The plant provides the fungus with carbohydrates. It is estimated that up to 20 % of the photosynthetic products of the plant can go to the fungal partner (Bago et al. 2000; Douds Jr. et al. 2000; Graham 2000). It is mostly transferred as glucose (Shachar-Hill et al. 1995; Schüßler et al. 2006; Nehls et al. 2010; Doidy et al. 2012), since it is shown by NMR spectrometry experiments that mycorrhizal intraradical hyphae cannot take up sucrose, but only hexoses as glucose, or in smaller amounts fructose (Solaiman & Saito 1997; Pfeffer et al. 1999). The fungus is also able to take up xylose as another carbon source (Schüßler et al. 2006; Helber et al. 2011). The hexoses are then converted to trehalose and glycogen (Shachar-Hill et al. 1995), and a considerable amount of hexoses is also used for the synthesis of storage lipids, which are transferred into the ERM and germinating spores (Pfeffer et al. 1999). As an obligate symbiont, the fungus relies on the carbon provided by the plant (Jennings 1995). In exchange, the fungus supplies the plant with water and nutrients such as phosphate, sulphate, and nitrogen (Egerton-Warburton et al. 2007; Javot, Penmetsa, et al. 2007; Casieri et al. 2012; Querejeta et al. 2003; Govindarajulu et al. 2005; Jin et al. 2005; Casieri et al. 2013). For the fungus, phosphate and other compounds are often easier to be taken up from soil than for the plant and the hyphae can explore a greater volume of soil than the roots. Furthermore, mycorrhizal fungi seem to have an overall positive effect on the host plants. The resistance to pathogens is increased, plant growth and reproduction are supported and the tolerance to abiotic stress can be enhanced due to the symbiosis (Valentine et al. 2001; Wu & Xia 2006; Van Wees et al. 2008; Liu et al. 2007).

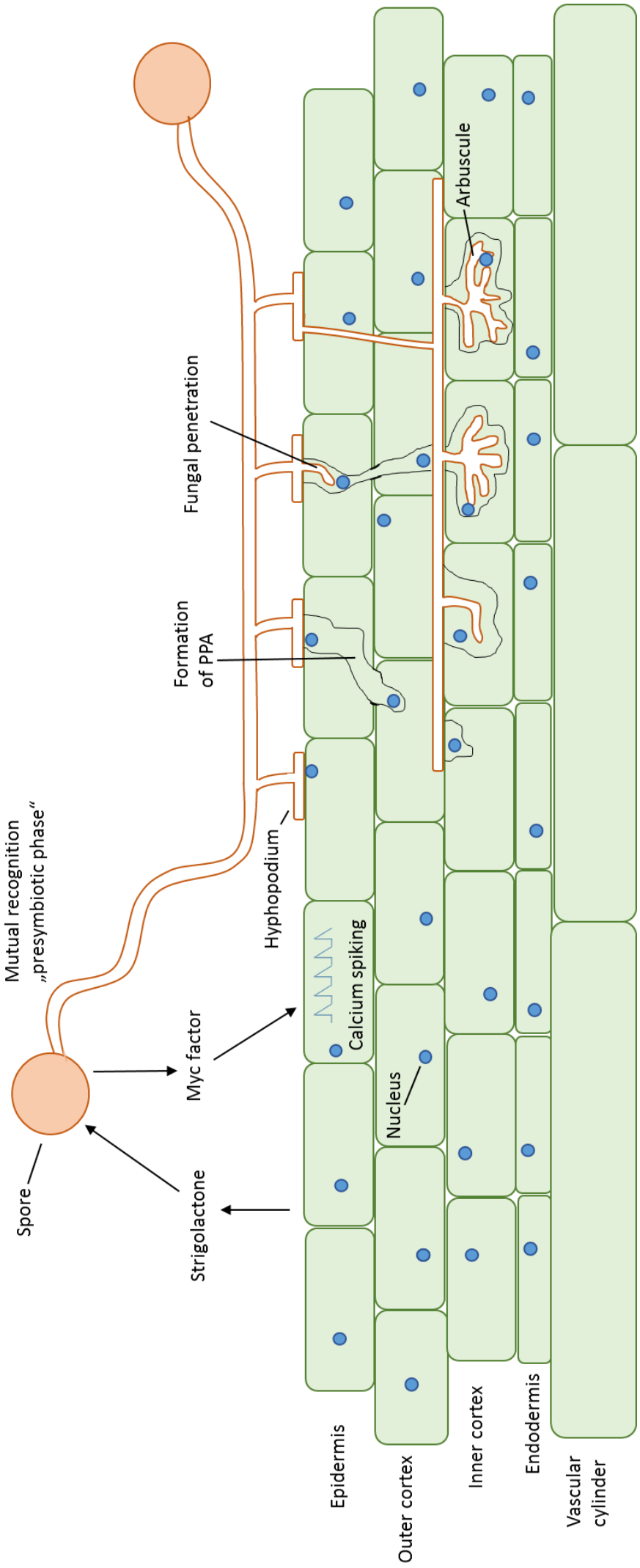

Figure I.1: Steps in the development of the arbuscular mycorrhizal symbiosis.

Plant cells are illustrated in green, fungal structures are illustrated in orange and white. The plant root releases Strigolactones. These function as a signal for spore germination and hyphal branching. As a further result, the fungus produces and releases mycorrhizal (Myc) factors, which in turn induce calcium oscillations in plant root epidermal cells. Symbiosis related genes in the plant cells are activated due to these signal molecules. The hypha forms the hyphopodium, a special kind of appressorium, on the outside of the plant root. As a reaction from the plant cell, the nucleus wanders to the cell wall, where the hyphopodium is located. A prepenetration apparatus (PPA) is formed within the plant cell. The hypha enters at the side of the PPA and is guided through the cells to the inner cortex. It leaves the plant cell and grows inside the apoplast along the root axis. Due to the presence of the hyphae, PPA-like structures are built in the inner cortical cells. The fungus can now enter these cells, and arbuscules are developed. In later stages, vesicles can be built as storage organs by the fungus. New spores are generally developed outside of the plant root. The Figure is adapted from Parniske 2008.

1.2 Phosphate Uptake and Transport in Plants With and Without Mycorrhization

Low phosphorus availability is an important growth limitation factor for plants (Marschner 1995). Orthophosphate (Pi) in soil solution is the only form of phosphorus directly accessible for the plant. However, in natural soils, phosphorus often occurs in organic forms as lecithin and phytate, often incorporated into large organic matter molecules, or as orthophosphates in stable complexes with iron or aluminum oxides/hydroxides (Holford 1997; Welp et al. 1983). As Pi is highly immobile in the soil, it is taken up faster by the roots than new Pi can reach it via diffusion, which creates a depletion zone around the roots and root hairs of the plant (Bieleski 1973; Tinker & Nye 2000). Plants have developed different strategies to overcome this problem. It was shown that root development is very sensitive to changes in the P distribution in soil (Wiersum 1958; Forde & Clarkson 1999; Forde & Lorenzo 2001). The plants react with changes in root architecture (López-Bucio et al. 2003), variations in the release of root secretions (Neumann et al. 2000), changes in Pi transport systems to increase P uptake under low concentrations, and the aforementioned mycorrhizal symbiosis to reach nutrients beyond the rhizosphere. The root architecture is regulated by genetic programming as well as biotic and abiotic influences from the surroundings (Schiefelbein & Benfey 1991; Aeschbacher et al. 1994; Lynch 1995; Zobel 1996). A very common response to low nutrient abundance, which occurs in most vascular plants, is the development of root hairs from root epidermal cells via tip growth (Gilroy & Jones 2000). The control of this process is genetic and several genes are involved (Parker et al. 2000). These structures increase the root diameter significantly and represent 70-90 % of the total root surface area (Bates & Lynch 1996). They have a considerable importance in nutrient acquisition as well as in water uptake (Gilroy & Jones 2000), formation of Rhizobium symbiosis (Kalsi & Etzler 2000; Cullimore et al. 2001) and anchoring of plants in the soil (Bailey et al. 2002). Studies have shown that root hair density and elongation increase in response to P deficiencies and both factors correlate with Pi acquisition efficiency (Bates & Lynch 1996; Ma et al. 2001; Schmidt & Schikora 2001; Zimmermann et al. 2003; Narang et al. 2000), whereas high P abundance can lead to absent or rudimentary root hairs (Föhse & Jungk 1983; Bates & Lynch 2000; Gahoonia et al. 2001).

However, for the actual uptake of Pi through the cellular plasma membrane, a transport system at the root-soil interface is needed. The membrane serves as a hydrophobic barrier between two hydrophilic sites. Therefore, a biochemical gradient as well as an electrochemical gradient can be build up between these sides. A proton gradient is reached with a P-type H^+-ATPase pump using ATP as an energy source (Ullrich-Eberius et al. 1984; Thibaud et al. 1988; Daram et al. 1998; Sze et al. 1999; Karandashov & Bucher 2005). Then, Pi and other anions can be cotransported with protons using the gradient as a driving force. For the transport of Pi, three families of transporters have been classified in plants, Pht1, Pht2 and Pht3 (Bucher et al. 2001; Mudge et al. 2002; Poirier & Bucher 2002; Rausch & Bucher 2002), with the proteins of the Pht1 family seeming to have the most

important role in P uptake. All Pht1 transporters can be assigned to the phosphate:H^+ symporter family and are secondary transporters (Bucher 2007). Their gene expression is very high in root cells, including root hair cells, the root cap, and the outer cortex (Daram et al. 1998; Liu et al. 1998; Chiou et al. 2001; Karthikeyan et al. 2002; Mudge et al. 2002; Schünmann et al. 2004), but can also be found in leaves and pollen. Therefore, extended functions of Pht1 proteins, as the Pi uptake in the elongation pollen tube or the remobilization of stored Pi through the phloem, seem possible (Rae et al. 2003; Mudge et al. 2002). The regulatory elements identified in Pht1 protein promoters suggest their expression can be precisely adjusted according to internal and external P condition. It is believed that Pi transporter regulation is primarily transcriptionally controlled, but post-transcriptional and post-translational modifications of regulatory elements might also play a role (Bucher 2007). The protein itself can be found dominantly in the plasma membrane of root cells, with an increasing amount in the tip of root hairs (Chiou et al. 2001; Gordon-Weeks et al. 2003). The phosphate uptake with these transporters is called the "direct P uptake pathway" (Bucher 2007).

As mentioned above, another plant strategy to improve Pi uptake is the symbiosis with a mycorrhizal fungi. This is called the "mycorrhizal uptake pathway"(Bucher 2007). The mycelium of the fungi can grow much further than the root hairs. It branches and can explore a greater volume of soil. Also hyphae are thinner than roots, which maximizes their interface with the soil and increases the volume of soil solution available for uptake (Jakobsen et al. 1992; Bieleski 1973). Mycorrhizal hyphae provide an effective network for Pi uptake outside of the depletion zone around the roots. Furthermore, ectomycorrhiza have the ability to mineralize organic P and thus make it available for plants (Smith & Read 2008). According to Koide and Kabir (2000), Joner, Ravnskov, and Jakobsen (2000) and Feng et al. (2003), arbuscular mycorrhiza (AM) might also have this ability. Moreover, early studies with ectomycorrhiza suggest that it is even possible for these fungi to increase the phosphate availability of mineral-bound phosphorus (Smith & Read 2008), and therefore perform biogenic mineral weathering. It is currently unclear if this is also possible for AM fungi (Koele et al. 2014).

The extraradical mycelium of the mutualistic symbiont takes up Pi with a specialized phosphate transporter (Maldonado-Mendoza et al. 2001) and transports it, mainly in form of polyphosphates, through the hyphae to the arbuscules inside of the plant cells. Here, it is taken up by the plant by mycorrhiza induced P transporters located in the periarbuscular membrane (Smith & Smith 1997; Harrison et al. 2002). Many such mycorrhiza induced or upregulated transporters have been described in recent years. One of the first was *MtPT4* in *Medicago truncatula* (Harrison et al. 2002). It is exclusively expressed during AM symbiosis, the protein is located in the periarbuscular membrane of the plant, and it seems to be crucial for maintaining the symbiosis (Javot, Penmetsa, et al. 2007; Harrison et al. 2002). Orthologues from several different species were identified in the following

years, including *OsPT11* in rice (Paszkowski et al. 2002), *HORvu:Pht1;8* in barley, *TRIae;Pht1;myc* in wheat, *ZEAma;Pht1;6* in maize (Glassop et al. 2005), and *LjPT3* in *Lotus japonicus* (Maeda et al. 2006). Two other examples are *StPT4* and *StPT5* from potato and their orthologues *LePT4* and *LePT5* from tomato (Nagy et al. 2005). The important role of *LePT4* was further verified by Xu et al. (2007). The functional importance for mycorrhization of most of these genes has been shown by many studies. For example, silencing of *MtPT4* expression leads to failure to increase Pi uptake in mycorrhized plants and the premature death of arbuscules (Javot, Penmetsa, et al. 2007). A split root experiment with *M. truncatula* demonstrated that only mycorrhized roots expressed the *MtPT4* gene whereas the non-mycorrhized side showed no expression (Liu et al. 2007). Therefore, expression rate of *MtPT4* and its homologues are commonly used as indicators for the level of mycorrhization (Javot, Pumplin, et al. 2007). It has also been shown that the phosphate transporters are exclusively expressed in arbusculated cells (Balestrini et al. 2007; Gómez-Ariza et al. 2009), which further verifies their significant role in mycorrhizal Pi uptake. In addition, several studies found an upregulation or accumulation of H^+-ATPases in mycorrhized root tissue (Murphy et al. 1997; Bago et al. 1997; Benabdellah et al. 1999). These genes are necessary to generate the proton gradient as an energy source for the Pi uptake over the periarbuscular membrane, and early studies already showed that H^+-ATPases are active at the periarbuscular membrane and are therefore involved in the active uptake of nutrients at the symbiotic interface (Marx et al. 1982; Gianinazzi-Pearson et al. 1991). Figure 1.2 shows the interaction of these proteins in an arbusculated cell. One of these H^+-ATPase proteins could be found in the plant membrane around the arbusculated hyphae in tobacco plants (Gianinazzi-Pearson et al. 2000) and the gene expression of a similar gene in *M. truncatula* (*Mtha1*) was shown to be activated during AM symbiosis exclusively in arbusculated root cells (Krajinski et al. 2002; Manthey et al. 2004). In tomato, the plasma membrane H^+-ATPase LHA2 was found to be upregulated in response to mycorrhization in the roots as well as in the leaves (Ferrol et al. 2002). Simultaneously, the H^+-ATPase LHA1 was downregulated due to mycorrhization. It is therefore likely that LHA1 is a component of the direct P uptake pathway, which is sometimes downregulated during AM symbiosis (Pearson & Jakobsen 1993), whereas LHA2 seems to be a part of the mycorrhizal P uptake pathway (Ferrol et al. 2002). The upregulation of genes as the aforementioned H^+-ATPases by mycorrhization shows that not only specialized P transporters are involved in the mycorrhizal P uptake pathway. It has also been shown that the P uptake derived from AM symbiosis can dominate the total P uptake in a plant even if the plant shows no positive growth response or increase in total P acquisition. Furthermore, a great variance in the contribution of the mycorrhizal uptake pathway in the total P uptake is possible, depending on the fungal and plant species involved in the symbiosis (Smith et al. 2003; Smith et al. 2004).

1.3 Mycorrhizal Phosphate Uptake from Organic Compounds and Minerals

The mechanism of P acquisition from phytate and other organic compounds has been discussed in several studies, but remains unclear. It was hypothesized that AM fungi (AMF) profit from other microorganisms in the soil, which hydrolyse organic compounds. This was supported by studies showing that AMF can influence the growth and behavior of other microorganisms in the soil. The influence can be indirect by modifying root exudates, or direct by the excretion of organic compounds (Linderman 1992; Fitter & Garbaye 1994; Toljander et al. 2007). The growth-promoting effect of AMF especially on phytase-producing bacteria (PSB) has recently been demonstrated again (Zhang et al. 2014; Zhang et al. 2016). The study comes to the conclusion that PSB and AMF provide carbon and inorganic phosphate for each other and can both benefit from their interaction. Another theory was that AMF can directly hydrolyse organic compounds without the aid of other organisms. Tarafdar & Marschner (1994) suggested that the extracellular acid phosphatase activity, which correlated with hyphal density, originated from the AMF itself. This theory was supported by two in vitro studies with carrot roots mycorrhized with *R. irregularis*, which both proved that extraradical AM fungal hyphae can hydrolyse organic P compounds and transport the obtained phosphate back to the plant (Koide & Kabir 2000; Joner et al. 2000). Both suggest an enzymatic hydrolysation, either by surface-bound or freely released phosphatases, with wall-bound phosphatases as the more likely alternative. The enzymes currently under discussion for the direct AM fungal P acquisition from organic sources like phytate are either acid phosphatase (Wang et al. 2013; Zhang et al. 2014; Saito 1995; Joner et al. 2000; Tarafdar & Marschner 1994; Joner & Johansen 2000) or phytase (Wang et al. 2017). Recent research suggest that phytase is presumably more important for the mineralizing of phytate (Wang et al. 2017). In maize inoculated with *Funneliformis mosseae* or *Claroideoglomus etunicatum*, a positive correlation between phytase activity and P-uptake could be observed, which was not the case for acid phosphatase (Wang et al. 2017).

Direct nutrient uptake from rock-forming minerals by ectomycorrhizal fungi (EMF) has been shown in several studies (Leyval & Berthelin 1989; Paris et al. 1996; Wallander et al. 1997; Glowa et al. 2003), as well as “tunnel” formations in minerals in close contact with ectomycorrhizal hyphae (Leake et al. 2008; Bonneville et al. 2009). Though not in the focus of research, there is evidence that AMF are also able to contribute in mineral weathering. Already in 1982, an experiment from Berthelin & Leyval (1982) with maize showed a promoting effect of AMF and non-symbiotic rhizosphere bacteria on mineral weathering of biotite. More recent research gives fossil evidence for AM mineral weathering of biotite in Miocene paleosols (Sanz-Montero & Rodríguez-Aranda 2012), and an experiment with potted plants and biotite as a potassium source also showed the potential of AMF in weathering (Arocena et al. 2012). A study by Quirk et al. (2012) further confirmed a role of AMF in mineral weathering of basalt and muscovite, though they suggested slower weathering rates for AMF

compared to EMF. Furthermore, Koele et al. (2014) showed the weathering of apatite particles by EMF, as well as AMF in natural environments in New Zealand. Nylon mesh bags with either reactive rock phosphate or crystalline apatite were buried in field sites either dominated by ectomycorrhizal or arbuscular mycorrhizal plant species. Fungal hyphae as well as linear features (called “tunnels”) were found in mineral grains or soil particles and aggregates for both sorts of field sites, suggesting mineral weathering by both fungal types. Additionally, the uptake of rare earth elements from bags from both field type sites showed no differences, further affirming this theory and suggesting no major difference in the P uptake of EMF and AMF from mineral sources. It is believed that the “tunneling” occurring in minerals under ectomycorrhizal influence is based on the secretion of organic acids. Studies show that organic acids enhance the release of phosphate from apatite, chalcopyrite, and other minerals (Arbel et al. 1991; Welch et al. 2002; Goyne et al. 2006; Sagoe et al. 1998; Nakamaru et al. 2000; Johnson & Loeppert 2006). Therefore, it seems possible that organic acids are also involved in AMF weathering. It has been demonstrated that AMF are able to excrete organic acids (Tawaraya et al. 2006; Toljander et al. 2007), which supports this mechanism. It is also under discussion that the frequently observed tunnels in minerals derive either from saprophytic fungi by direct tunnel formation or by acidifying topsoil through the decomposition of organic matter, or that tunnels form, independent from biological interactions, by combined acidification of the soil biotic agents and also organic matter decomposition (Sverdrup 2009). Though a direct P uptake from the apatite by the fungus is possible, it can also not be excluded that a general soil acidification by different microorganisms leads to the release of phosphate and other earth elements from the minerals. The hyphae would then just take up the elements from the soil solution. Koele et al. (2014) suggests that general mycorrhizosphere acidification is the most likely mechanism for nutrient uptake by AMF. To fully understand the processes, more research in this field is still necessary.

1.4 Mycorrhiza Induced Sucrose Synthase and Sugar Transporter Genes in Plants

Sugar transporters in plants, including sucrose (SUTs) as well as monosaccharide (MSTs) transporters, are the key components in the long distance transport of sugars in the phloem from source to sink organs. Both groups belong to the major facilitator superfamily and are supposed to be H^+/sugar symporters with 12 transmembrane domains connected by hydrophilic loops. They mediate the transport starting in the mesophyll cells, where the newly synthesized sucrose is loaded into the collection phloem. From there, it is transported through the transport phloem into the roots, where it is unloaded into the release phloem and eventually into the roots for storage, as an energy source, or for trading with microorganisms (Doidy et al. 2012). Although the role of sugars as highly energetic

metabolites is obvious, they are also important signaling molecules. The whole transport system is crucial for functions as cell to cell communication, environmental adjustment, plant maturation and regulation of carbon partitioning (Rolland et al. 2006).

It has been shown that the roots of mycorrhizal plants also act as an important sink system for photosynthetic products, as the colonization by heterotrophic organisms increases the sink strength (Wright, Scholes, et al. 1998; Wright, Read, et al. 1998; Doidy et al. 2012; Black et al. 2000). Mycorrhizal plants make up for the higher carbon costs by an increased CO_2 assimilation and higher photosynthetic rate in the leaves (Wright, Scholes, et al. 1998; Boldt et al. 2011; Doidy et al. 2012), which leads to an increased carbohydrate flux through the system. This is accompanied by an upregulation of genes encoding for hexose transporters, invertases and sucrose synthases [for review: (Doidy et al. 2012)]. For example, in *M. truncatula* roots colonized by AM fungi, the sugar transporter Mtst1, which transports glucose and fructose, shows an increased expression in mycorrhized plants, especially in phloem fiber cells of the vascular tissues and in colonized root tip cells (Harrison 1996). Furthermore, the sucrose synthase gene *MtSucS1* is highly activated in cortical cells of AM colonized roots. The study suggests that it is involved in generating sink-strength in the roots (Hohnjec et al. 2003). The essential role of MtSucS1 for an effective mycorrhization and especially for the arbuscule formation and maintenance was confirmed via experiments with knockdown mutants of *M. truncatula* (Baier et al. 2010). In maize, the sucrose synthase genes *Sus1* and *Sh1* were upregulated in roots colonized with three different arbuscular mycorrhizal fungal isolates. Their regulation was also influenced by the P supply of the plant, but contrary to the mycorrhizal impact (Ravnskov et al. 2003). The upregulatory effect of the mycorrhization decreased as the plant grew older and the shoot growth transgressed that of the non-mycorrhizal control plants. As an explanation, the study suggest that the C allocation to the fungus lessens with the proceeding establishment of a successful colonization (Ravnskov et al. 2003). In a study with *Lycopersicon esculentum* colonized with *Rhizophagus irregularis*, the expression of the apoplastic invertase LIN6 was significantly upregulated in colonized cells and the central cylinder of root tissue compared to non-mycorrhized plants (Schaarschmidt et al. 2006). Interestingly, a wounding of non-mycorrhized tomato plants led to an even higher increase in *LIN6* expression. It was presumed that the response in expression of *LIN6* to external factors can be precisely regulated to avoid stress responses in the mycorrhizal interaction (Schaarschmidt et al. 2006). LIN6 is known to play a key role in establishing and maintaining sink metabolism. As an invertase, it cleaves sucrose into the two hexoses fructose and glucose. Another study with tomato and the mycorrhizal fungi *Glomus mossae* showed that mycorrhized plants assimilated a significantly higher amount of CO_2 than unmycorrhized plants, combined with heightened amounts of sucrose and fructose levels in the roots. Glucose levels were not changed, which indicates that the glucose generated from the sucrose decomposition was

transferred to the fungal symbiont. As no sucrose cleaving activity has been observed so far in mycorrhizal fungi, the sucrose has to be cleaved by plant enzymes like apoplastic and symplastic invertases (Schubert et al. 2004; Sturm 1999). In addition, the three sucrose transporter encoding genes *SlSUT1*, *SlSUT2* and *SlSUT4* were upregulated in leaves and in part in root tissue of mycorrhized tomato plants, supposedly to provide for the higher demand in sucrose transport (Boldt et al. 2011). In contrast, an earlier study with tomato found no change in expression of *SlSUT2* and *SlSUT4* due to mycorrhization, but a downregulation of *SlSUT1* (Ge et al. 2008). In potato, an overexpression of the analogue transporter *SoSUT1* resulted in an increase of mycorrhization under high P conditions (Gabriel-Neumann et al. 2011). A role of *SUT1* in mycorrhization can therefore not be excluded. The mycorrhiza-related function of *SlSUT2* could also be confirmed (Bitterlich et al. 2014). A down-regulation of the gene in the roots led to an increase in mycorrhization and dry weight of extraradical mycelium, whereas the positive growth response of the plant as well as the normally increased photosynthetic parameters were abolished (Bitterlich et al. 2014). Further experiments showed that the SlSUT2 protein is supposedly located in the periarbuscular membrane, while SlSUT1 and SlSUT4 could not be found in the periarbuscular membrane or in arbusculated cells in general. This finding implies a major role of *SlSUT2* in mycorrhizal sugar transport compared to the other potential transporters (Bitterlich et al. 2014). In addition, the SlSUT2 protein was shown to interact with proteins of the brassinosteroid signaling or biosynthesis pathway and thus a role of brassinosteroids in mycorrhizal function and development seems possible. Figure I.2 shows the general interaction of several plant proteins involved in the mycorrhizal symbiosis in an arbusculated cell.

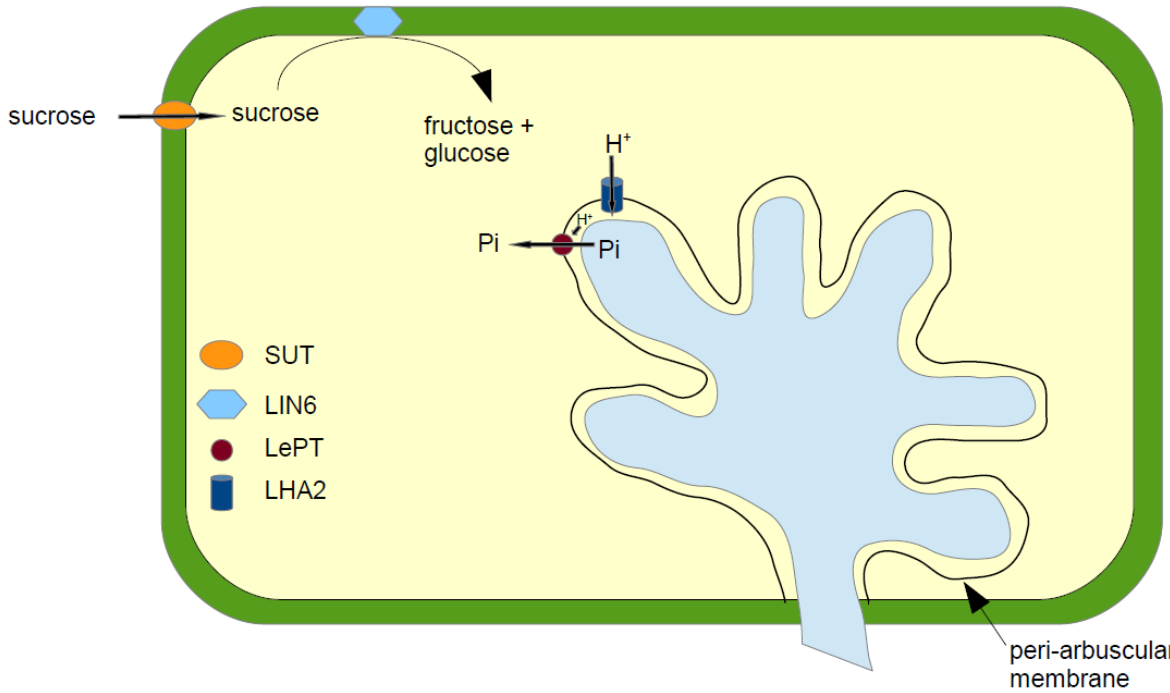

Figure I.2: Schematic picture of the main proteins involved in the sugar and phosphate transport in the mycorrhizal symbiosis. The apoplastic cell membrane is illustrated in green, the fungal arbuscule in light blue. SUT is a sucrose transporter, LIN6 is an apoplastic invertase, LHA is an H^+-ATPase located in the periarbuscular membrane, LePT is a mycorrhizal phosphate transporter located in the periarbuscular membrane.

1.5 Fungal Transporter Genes in the Mycorrhizal Symbiosis

Although the research is not yet as extensive as in the plant symbiont, there are also many transporters involved on the fungal side of the symbiosis, including phosphate and sugar transporters. In the majority of AM fungal species, it was not possible to detect the activity of invertases (Schubert et al. 2004). Therefore, it is assumed that the sucrose breakdown is mainly catalyzed by the plant itself before fungal uptake of the carbohydrates (Doidy et al. 2012). However, monosaccharide transporters (MSTs) can be found in different fungal species, including ecto- and endomycorrhiza. The first characterized MSTs from ectomycorrhizal fungi have been AmMST1 and AmMST2 from *Amanita muscaria* (Nehls et al. 1998; Wiese et al. 2000; Grunze et al. 2004) and TbHXT1 from *Tuber borchii* (Polidori et al. 2007). For all three transporters, glucose is the preferred substrate (Nehls et al. 1998; Grunze et al. 2004; Polidori et al. 2007). The expression of *AmMST1* and *AmMST2* is increased by higher external sugar concentrations and in ectomycorrhizal symbiosis compared to the extraradical mycelium. Therefore, it was postulated that these transporters take up carbohydrates at the symbiotic interface (Nehls et al. 1998; Grunze et al. 2004). However, *TbHXT1* expression is enhanced by carbohydrate starvation instead, indicating a role in the uptake of carbohydrates by soil-growing hyphae (Polidori et al. 2007). Another important ectomycorrhizal fungus, *Laccaria bicolor*, contains 15 putative MSTs in its genome (López et al. 2008). For most, glucose could also be characterized as their preferred substrate over fructose. Different functional experiments have been made and the MSTs of *L. bicolor* could be divided into two main groups based on their putative function, with one group responsible for sugar uptake at the plant-fungal interface and the other group for the carbohydrate uptake from the soil to improve carbon nutrition and reduce nutrient uptake competition with other soilborne organisms (López et al. 2008).

For the AM fungi, the GpMST1 was isolated as the first symbiosis-related MST from *Geosiphon pyriformis* in symbiosis with *Nostoc punctiforme* (Schüßler et al. 2006). Although the symbiotic partner of the fungus is a cyanobacterium rather than a plant, the attained inside in the genomic data helped to identify other MSTs in *Glomus sp*. In the model species *R. irregularis*, the versatile monosaccharide transporter *GiMST2*, which is a high affinity H^+/glucose transporter, plays an important role in the carbon transfer during the symbiosis (Helber et al. 2011). It is expressed in arbuscules as well as in the intercellular mycelium. Two other MSTs, *GiMST3* and *GiMST4*, were also identified, but their expression was comparably low in the in planta phase of the symbiosis. Besides, a silencing of *GiMST2* led to malformed arbuscules, impaired mycorrhizal formation and an increase in the expression of the plant phosphate transporter *MtPT4* in *M. truncatula*. Therefore, GiMST2 is believed to be the primary sugar uptake transporter in this species (Helber et al. 2011). The expression of *GiMST2* correlates with the expression of the plant phosphate transporter gene *PT4* and an increase in phosphate availability led to a significant down-regulation of both transporters in

an in vitro experiment with mycorrhized potato roots, which indicates a link between *MST2* expression and symbiotic phosphate delivery (Helber et al. 2011). Although GiMST2 has a high affinity for glucose, it is also able to bind xylose and other plant cell wall monosaccharides, which were even able to outcompete glucose uptake. Thus, it was hypothesized that cell wall monosaccharides can also be utilized as a carbon source for AM fungi (Helber et al. 2011). Additionally, xylose can also induce the expression of *GiMST2* in the extraradical mycelium (Helber et al. 2011), which is currently believed to be unable to take up sugars (Pfeffer et al. 1999). Further experiments with radioactive glucose and xylose showed that an uptake of both substances from the mycelium is at least in vitro indeed possible (Helber et al. 2011). In 1995, the phosphate transporter GvPT from the AM fungus *Glomus versiforme* was cloned and characterized (Harrison & van Buuren 1995). The phosphate uptake capacity was experimentally confirmed and the data indicate a proton-coupled symport of phosphate. GvPT is expressed in the extraradical hyphae and also in extremely low amounts in the fungal structures inside of the roots, suggesting a main role in the uptake of phosphate from the soil into the extraradical hyphae (Harrison & van Buuren 1995). Furthermore, the high affinity phosphate transporter GiPT was identified in *Rhizophagus irregularis* (Maldonado-Mendoza et al. 2001). Its expression seems to be regulated by the amount of phosphate present in the substrate surrounding the extraradical mycelium, regardless of the symbiotic partner. In root culture experiments, the expression of *GiPT* was nearly zero if no phosphate was available, it increased if phosphate concentrations were similar to concentrations in natural soils (5-35 µM), and a decrease in expression could be observed under high phosphate conditions (3.5 mM). Moreover, a sufficient phosphate status of the mycorrhized roots led to a lower induction of *GiPT* in the extraradical mycelium in response to phosphate addition than observed for roots formerly deprived of phosphate (Maldonado-Mendoza et al. 2001).

1.6 Phospholipid Fatty Acids and Neutral Lipid Fatty Acids in Plants and Mycorrhizal Fungi

Lipids play an important role in living organisms, and various types can be found in plants, fungi and bacteria. Two fundamental types are phospholipids, which are the primary membrane constituents, and neutral lipids, which are essential to store carbohydrates and therefore energy in eukaryotes. Lipids typically consist of hydrophobic fatty acids connected to a hydrophilic glycerol backbone. In phospholipids, two fatty acids are connected to phosphate via the glycerol molecule. Storage lipids contain three fatty acids and are therefore triacylglycerines or triacylglycerides (TAG). The biosynthesis of fatty acids in animals and fungi takes place primarily in the cytoplasm, whereas plants produce fatty acids mainly in the plastids (Ohlrogge & Browse 1995). The basic fatty acids in plants are 16-carbon palmitate and 18-carbon oleate, linoleate, and α-linolenate. The carbon necessary for

their synthesis derives from the pool of acetyl-coenzymeA (CoA) present in the plastids (Harwood 1996). In the first step, malonyl-CoA is formed from Acetyl-CoA and CO_2 with the help of Acetyl-CoA carboxylase (ACCase) and ATP as an energy-source. Then, the malonyl group is transferred to the acyl carrier protein (ACP). This protein cofactor is now involved in every step of the synthesis. A four carbon chain is formed by the condensation of malonyl-ACP and Acetyl-CoA catalyzed by one of three condensing enzymes called 3-ketoacyl-ACP synthases. Elongation goes on until C16 or C18 chains (palmitoyl-ACP or steroyl-ACP) are synthesized and it is terminated with the removal of the acyl group from ACP (Ohlrogge & Browse 1995; Harwood 1996). The synthesis products are either directly used for membrane lipid synthesis or exported into the cytoplasm. They are further processed at the chloroplasts or the endoplasmatic reticulum to produce storage lipids, membrane lipids or polyunsaturated fatty acids like linoeic or linolenic acid (Browse & Somerville 1991). The synthesis of fatty acids in AM fungi follows the same basic patterns. Fatty acids are then elongated, desaturated and esterified with a glyceryl moiety to produce storage lipids (Pfeffer et al. 1999; Bago et al. 2000). The synthesis of TAG represents a major sink for carbon in the intraradical hyphae and TAG are the main storage form of fungal carbohydrates (Pfeffer et al. 1999; Bago et al. 2000; Beilby & Kidby 1980; Jabaji-Hare 1988; Lösel & Cooper 1979). The carbon necessary for the production of fatty acids are host-derived. Hexoses are then metabolized by the fungus to triose and Acetyl-CoA via glycolysis or converted into trehalose and glycogen, which are also substantial fungal C pools and the characteristic fungal carbohydrates (Shachar-Hill et al. 1995). The following synthesis of storage lipids takes place exclusively in the IRM. As shown by ^{13}C labeling and nuclear magnetic resonance spectroscopy, the ERM is not able to take up exogenous hexoses and any indication for the production of storage lipids in the ERM could not be observed so far (Pfeffer et al. 1999; Lammers et al. 2001). Furthermore, no or nearly no glycolytic enzyme activity could be found in the ERM (MacDonald & Lewis 1978; Saito 1995). However, a considerable gluconeogenic flux exists in the ERM and the functioning of the glyoxylate cycle as well as the pentose phosphate pathway could be demonstrated (Saito 1995; Pfeffer et al. 1999; Lammers et al. 2001). The data suggests that gluconeogenic C fluxes are fueled in the ERM by metabolism of TAG and the needed carbon derives from TAG catabolism and imported glycogen. Both glycogen and TAGs thus have to be transported from the IRM to the ERM. Figure I.3 gives an overview of the carbon metabolism and transport in AM fungi. Lipid bodies, also called oleosomes, build by the fungi, can be found in arbuscular trunks, inter- and extracellular hyphae, extraradical spores and germ tubes (Bonfante et al. 1994). The movement of large quantities of these lipid bodies in “runner hyphaes”, which act as conduits for nutrient translocation, is maintained via cytoplasmic streaming (Bago, Zipfel, et al. 2002). Since the AM fungal hyphae of *Glomus sp*. carry large amounts of lipids, they can be seen as “oleogenic” fungi, which can store 25 % of their dry weight as lipids (Jabaji-Hare 1988).

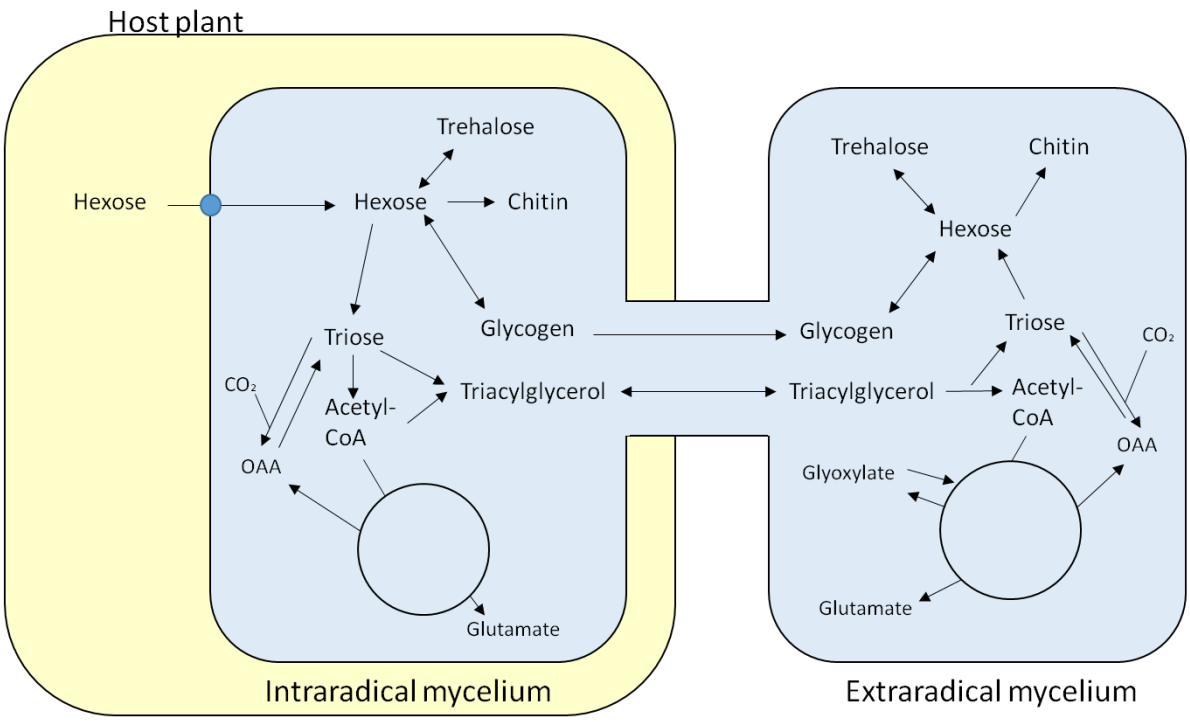

Figure I.3: Schematic overview of the carbon metabolism and transport processes in AM fungi. Carbon is provided as hexose by the host plant and converted into trehalose, chitin, glycogen and storage lipids in the IRM. Glycogen and lipids are transported into the ERM, where they can be further metabolized. The figure is adapted from Bago et al. 2000; Bago et al. 2002; Bago et al. 2003

The fatty acids of phospholipids (PLFAs) and neutral lipids (NLFAs) can be used to determine the microbial structure in soil samples, to estimate the biomass of AM fungi in soil or roots, and to evaluate the carbon allocation inside mycorrhizal hyphae (Olsson et al. 1995; Olsson 1999). This is mainly possible due to the varying composition of fatty acids in different organisms. Furthermore, PLFA-based measuring methods have the advantage of depicting the appearance of living or just recently dead organisms, since the phosphate group of phospholipids is quickly released through enzymatic actions in the surroundings (White et al. 1979). Together with NLFA measurements, it is possible to estimate the nutritional status of a eukaryotic organism through the neutral- to phospholipid ratio (Tunlid & White 1990). In AM fungi, a major amount of the total fatty acids consists of 16:1ω5 and 18:1ω7 (Beilby 1980; Nordby et al. 1981; Pacovsky & Fuller 1988; Nakano et al. 2001; Pearson & Jakobsen 1993). For example, the total amount of NLFA of *R. irregularis* contains 50 – 70 % of the fatty acid 16:1ω5 (Olsson & Johansen 2000), differentiating them from other fungi, where these two fatty acids are not typical (Müller et al. 1994). However, they can be present in some bacterial genera (Olsson et al. 1995), which is why it may be necessary to use additional techniques for identification, depending on the sample composition. One complementary criteria could be the content of polyunsaturated 20-carbon fatty acids, as AM fungi have a rather high

content compared to non-AM fungi or bacteria, though they are also present in algae and protozoa (Graham et al. 1995; Johansen et al. 1996; Lechevalier & Lechevalier 1988; Federle 1986).

Furthermore, it can be possible to determine between different AM fungal species and genera, as there are considerable differences between their fatty acid composition (Lechevalier & Lechevalier 1988). With the analysis of PLFA 16:1ω5, the AM fungal biomass in soil as well as root samples can be estimated and compared between samples (Olsson et al. 1998; Olsson et al. 1995; Olsson et al. 1997). With the specific fatty acids from PLFA and NLFA measurement, the relation between biomass of mycelium and storage structures can be evaluated and hence the amount of carbon allocation to the fungi can be estimated (Peng et al. 1993; Olsson et al. 1997). It is a useful tool to determine the functional status of a symbiosis and the trading mechanisms of both partners.

1.7 Perspective of the Thesis

In the aforementioned studies on the influence of mycorrhization on the regulation of phosphate transporter expression, environmental factors such as the phosphate status of the surrounding soil or the source of phosphate available to plant and fungus, have not been taken into account. However, in nature phosphate is available in many different forms and not evenly distributed in the soil. Therefore, this thesis should help determine potential links between phosphate availability and accessibility in the substrate and the expression rate of sugar and phosphate transporters involved in the mycorrhizal symbiosis on the plant and fungal side. For this purpose, three experiments with two different experimental set-ups and two plant species were conducted. At first, a split root experiment with tomato plants and the mycorrhizal fungus *Rhizophagus irregularis* was constructed, in which only one side of the roots and hyphae had access to a phosphate source, whereas the other side received no phosphate at all. Two experiments were carried out in this design. In the first one, a fertilization with phosphate solution was compared to no phosphate fertilization. In the second split root experiment, apatite was added as a less accessible phosphate source and compared to either a fertilization with phosphate solution or no phosphate fertilization. My main interest lied in the phosphate transporters *LePT4* and *LePT3*, which are both upregulated during the mycorrhizal symbiosis, due to their direct involvement in the phosphate transfer from the fungus to the plant. Therefore, *LePT4* is a common marker for the degree of mycorrhization. I hypothesized that the phosphate availability and accessibility have an influence on the phosphate transporter expression and that a lower phosphate availability results in a lower expression level of the plant phosphate transporters. Since the fungus receives sugar in exchange for the delivered phosphate, expression of the sugar transporters might be linked to the phosphate transport from the fungus to the plant and

consequently, lower phosphate availability might lead to a downregulation of the transporters involved. Therefore, expression levels of the two sugar transporters *SlSUT1* and *SlSUT2* from tomato, which are known to be upregulated in mycorrhized roots or leaves, were also analyzed. Here, I expected a downregulation of both sugar transporters, if less phosphate was delivered to the plant. Finally, expression levels of the main sugar and phosphate transporters of *Rhizophagus irregularis*, *GiPT* and *GiMST2*, were analyzed to determine if their expression is influenced by the phosphate availability in the surrounding soil as well. Looking into the transporters on the fungal side additionally to the transporters on the plant side might give more information about the controlling processes in the symbiosis. I hypothesized a downregulation of both fungal transporters, if less phosphate was available to the fungus.

To address the question if the mycorrhizal plant changes the amount of carbon invested to the fungus, depending on the accessibility of the nutrient source available to the fungus, I designed a second experiment. Mycorrhized tomato plants were fertilized with three different phosphate sources: apatite as a mineral source, phytate as an organic phosphate source and a phosphate solution. Only the hyphae had access to these phosphate sources. The experiment was carried out over a period of 24 weeks and 6 time points were chosen for analysis, to evaluate changes within the time period. The hypothesis was that the plant changes the carbon investment over time depending on the accessibility of the P source. Furthermore, I wanted to supplement my information from the first experiment about the expression patterns of the major phosphate transporters *LePT4* and *LePT3* as well as the sugar transporter *SlSUT2*. My hypothesis was that phosphate and sugar transporter expression changes according to the accessibility of the P source and that they are linked to the carbon investment of the plant. I theorized that a higher throughput of phosphate would be linked to an upregulation of the corresponding transporter and a higher C investment would be coupled with a higher sugar transporter expression. Both genes are currently candidates as a marker for phosphate and carbon trading in the mycorrhizal symbiosis and my experiments should help to evaluate their suitability.

The third experiment was a repetition of the split root experiment with *Medicago truncatula* as a host plant. I chose to introduce a second plant species to determine if the interactions between host and fungus observed in the first experiments are species dependent. Studies show a high functional diversity and differences in carbon partitioning in the mycorrhizal symbiosis between different plant and fungal species (Lerat, Lapointe, Piché, et al. 2003; Lerat, Lapointe, Gutjahr, et al. 2003; Munkvold et al. 2004; Gosling et al. 2013). A repetition of the first experiment with a second species therefore seemed sensible. My hypothesis was that the expression of the plant phosphate transporter *MtPT4* will decrease, if less phosphate is available to the plant or if the phosphate source is less accessible. The experiment could therefore further verify or refute the usage of *MtPT4* as a mycorrhizal marker.

To gain more insight in the carbon trading of the symbionts and to complement the data gathered in the second experiment, I additionally labeled one repetition in the experiments with *M. truncatula* with gaseous ^{13}C and performed a PLFA and NLFA analysis. I hypothesized that more carbon would be invested by the plant to gain the same amount of P, if the phosphate source was harder to access for the fungus.

2. Material and Methods

2.1 Material

2.1.1 Chemicals and Consumables

The commonly used chemicals had a purity of at least p.a., if not further specified in the method description. Chemicals were ordered at the following companies: AppliChem (Darmstadt, Germany), Carl Roth GmbH (Karlsruhe, Germany), Linde (Hanover, Germany), Merck KGaA (Darmstadt, Germany), Sigma Aldrich (St. Louis, Missouri, USA), VWR International GmbH (Darmstadt, Germany)

The commonly used consumables were ordered from the following companies: BioRad Laboratories GmbH (Munich, Germany), Brand (Wertheim, Germany), Eppendorf (Hamburg, Germany), Greiner (Solingen, Germany), Ortmann (Vlotho, Germany), Sarstedt (Nümbrecht, Germany), Sigma Aldrich (St. Louis, Missouri, USA), VWR International GmbH (Darmstadt, Germany), Whatman (Maidstone, UK).

2.1.2 Special chemicals and consumables

Table II.1 Special chemicals and consumables

Chemical	Manufacturer	Further information
Carrier material for control inoculation	Symplanta (Munich, Germany)	Attapulgit-clay based carrier in mineral powder
DNaseI	Invitrogen (Carlsbad, California, USA)	DNAse digestion, cDNA synthesis
dNTP mix	Thermo Fisher Scientific (Waltham, Massachusetts, USA)	cDNA synthesis
Germ Agglutinin, Alexa Fluor® 488 Conjugate (catalogue W11261)	Thermo Fisher Scientific (Waltham, Massachusetts, USA)	Staining of mycorrhizal roots
M-MLV reverse transcriptase	Promega (Mannheim, Germany)	cDNA synthesis
NucleoSpin® RNA Plant	Machery& Nagel (Düren, Germany)	RNA extraction from plant material
Phopshate standard solution	Merck KGaA (Munich, Germany)	Phosphate measurement
Platinum®SYBR® Green qPCR SuperMix-UDG with Rox	Invitrogen (Carlsbad, California, USA)	Quantitative PCR
Rhizophagus irregularis research grade (10.000 spores/g)	Symplanta (Munich, Germany)	*Rhizophagus irregularis* research grade (10.000 spores/g)
Sodium carbonate-^{13}C (99 atom % ^{13}C)	Sigma-Aldrich (Munich, Germany)	^{13}C label of plants

2.1.3 Equipment and instrumentation

Table II.2 Equipment and instrumentation

Instrument	Manufacturer	Application
ABI Prism®7300	Applied Biosystems (Foster City, California, USA)	Quantitative PCR
Agarose gel equipment	Biozym (Hessisch Oldenburg, Germany)	Agarose gel electrophoresis
Ball mill MM200	Retsch (Haan, Germany)	Milling plant and soil samples
Ball mill MM400	Retsch (Haan, Germany)	Milling plant and soil samples
BioTek Synergy MX	BioTek (Winooski, Vermont, USA)	Elisa-Reader
Cryofuge 6000	Heraeus (Hanau, Germany)	Centrifuge with cooling
EGM-4	PP systems (Amesbury, Massachusetts, USA)	CO_2 measurement
Eppendorf Mastercycler® Pro	Eppendorf (Hamburg, Germany)	PCR and cDNA synthesis
GC System, 7890 A	Agilent Technologies (Santa Clara, California, USA)	PLFA and NLFA measurement
ICP-MS 7500 CX	Agilent Technologies (Santa Clara, California, USA)	Nutrient analysis
INTAS Gel Imager	INTAS (Göttingen, Germany)	Documentation of agarose gels
Isoprime	Elementar (Hanau, Germany)	Stable Isotope Ratio Mass Spectrometer
Muffler oven thermicon P®	Heraeus (Hanau, Germany)	Incineration of plant material
Nikon Eclipse Ti fluorescence microscope with Nikon Plan Apo, 10x/0.45 objective	Nikon (Japan)	Fluorescence microscopy of AlexaFluor stained mycorrhized roots
Phytochamber	Johnson controls (Milwaukee, Wisconsin, USA)	Growing of *M. truncatula* plants
Rotanta 460	Hettich (Tuttlingen, Germany)	Centrifugation
Rotary evaporator Laborota 4000 efficient	Heidolph (Schwabach, Germany)	PLFA and NLFA analysis
Vario EL III	Elementar (Hanau, Germany)	CN analysis
Vario isotope cube	Elementar (Hanau, Germany)	Elemental analysis

2.1.4 Oligonucleotides

The oligonucleotides were ordered from Metabion (Planegg/Steinkirchen, Germany) and eurofins Genomics (Ebersberg, Germany).

Table II.3 Oligonucleotides used in PCR reactions

Gene/Name	Function	Sequence (for/rev)
LePT4	Mycorrhizal tomato phosphate transporter	GAAGGGGAGCCATTTAATGTGG/ CCTTCAATAATCGCAGTGTAAC
LePT3	Mycorrhizal tomato phosphate transporter	TGTTCGCGTTAGCCATACCA/ CTGATATCCCGTGGCACGTT
SlSUT1	Tomato sucrose transporter	TTCCATAGCTGCTGGTGTTC/ TACCAGAAATGGGTCCACAA
SlSUT2	Tomato sucrose transporter	GGCCTGCACCGCTATCATT/ GGTTACAGCAAGAGGAATGCC
LeTefα	Translation elongation factor EF-1α of tomato	TGGAACTGTGCCTGTTGGTC/ ACATTGTCACCAGGGAGTGC
GiPT	Phosphate transporter of *R. irregularis*	TGTCATGGTTCGCGTTGGAT/ CTGCATCATGTGTGTCAGCG
GiMST2	Monosaccharide transporter of *R. irregularis*	GGCAGGATATTTGTCTGATAG/ GCAATAACTCTTCCCGTATAC
GiTefα	Translation elongation factor EF-1α of *R. irregularis*	TGTTGCTTTCGTCCCATT/ GGTTTATCGGTAGGTCGA
GiRNS	5.8S gene of *R. irregularis*	GTATGCCTGTTTGAGGGTCAGTATT/ AAACTCCGGAACGTCACTAAAGAG
MtPT4	Mycorrhizal phosphate transporter of *M. truncatula*	TCGCGCGCCATGTTTGTTGT/ GCGAAGAAGAATGTTAGCCC
MtTefα	Translation elongation factor EF-1α of *M. truncatula*	AAGCTAGGAGGTATTGACAAG/ ACTGTGCAGTAGTACTTGGTG
Oligo $d(T)_{15}$	cDNA synthesis, priming to the poly(A) tail of mRNA molecules	5´-d (TTT TTT TTT TTT TTT)-3´

2.1.5 Frequently Used Buffers and Solutions

Table II.4 Buffers and solutions

Name	Components	Concentrations
Agarose gel buffer (6x)	Glycerin	60 % (v/v)
	EDTA pH 8	60 mM
	Tris-HCl pH 7,6	10 mM
	Orange G	0,03 % (v/v)
Bligh and Dyer solution	Chloroform ($CHCl_3$)	1 part
	Methanol (CH_3OH)	2 parts
	Citric buffer (pH 4)	0.8 parts
DNaseI buffer	Tris-HCl pH 7,5	0.2 M
	$MgCl_2$	20 mM
Hoagland nutrient solution	$Ca(NO_3)_2{\cdot}4H_2O$	2.5 mM
	KNO_3	2.5 mM
	$MgSO_4{\cdot}7H_2O$	1 mM
	KH_2PO_4/K_2HPO_4	20 µM/ 1 mM
	NaFeEDTA	20 µM
	$Na_2MoO_4{\cdot}2H_2O$	0.2 µM
	H_3BO_3	10 µM
	$NiSO_4{\cdot}6H_2O$	0.2 µM
	$ZnSO_4{\cdot}7H_2O$	1 µM
	$MnCL_2{\cdot}4H_2O$	2 µM
	$CuSO_4{\cdot}5H_2O$	0.5 µM
	$CoCl_2{\cdot}6H_2O$	0.2 µM
Long Ashton nutrient solution with and without potassium	$Ca(NO_3)_2{\cdot}4H_2O$	4 mM/ 4.67 mM
	KNO_3	4 mM/ 0 mM
	$MgSO_4{\cdot}7H_2O$	1.5 mM
	$(NaH_2PO_4)\cdot2H_2O$	0.32 mM/ 0 mM
	Fe(EDTA)	0.1 mM
	H_3BO_3	46 µM
	$MnSO_4$	10 µM
	$ZnSO_4{\cdot}7H_2O$	1 µM
	$CuSO_4{\cdot}5H_2O$	0.9 µM
	$(NH_4)6Mo7O_{24}{\cdot}4H_2O$	0.5 µM
MMLV buffer (5x)	Tris-HCl pH 8.3	50 mM
	MgCl2	7 mM
	KCl	40 mM
	DTT	10 mM
	BSA	0.1 mg/ml
	[^{3}H]dTTP	0.5 mM
	Oligo(dT)	0.025 mM
	Poly(A)	0.25 mM
	NP-40	0.01 %
PBS buffer (1x) pH 7,4	NaCl	137 mM
	KCl	2.7 mM
	KH_2PO_4	12 mM
Tris-actate EDTA buffer (TAE) (50 x)	Tris-HCl	2 M
	Ice-vinegar	5 % (v/v)
	EDTA pH 8	50 mM

2.1.6 Standard substances

Table II.5 Standard substances for the EA-IRMS measurement

Substance	Weight (mg)	Company
Quartz sand (Blank)	20	In-house standrad
High organic sediment (HOS)	20	IVA Analysetechnik (Meerbusch, Germany)
Cellulose	4.8	International Atomic Energy Agency, Seibersdorf Laboratory (Vienna, Austria)
Caffeine	4.2	International Atomic Energy Agency, Seibersdorf Laboratory (Vienna, Austria)
$CaCO_3$	16	In-house standard
Needle litter (Nadel)	5-10	In-house standard (Waldstein, Germany)
Acetanilide	2-2.5	Merck KGaA (Darmstadt, Germany)

Table II.6 Standard Substances for NLFA measurement

Substance	Predominant origin	Reference
14:0		
i15:0		
a15:0		
15:0		
i16:0		
16:1ω7c	AM fungi	(Olsson 1999)
16:1ω5c	AM fungi	(Olsson et al. 1995; Olsson 1999)
10Me16:0		
i17:0		
a17:0		
17:0		
18:2ω6,9	Fungi (saprophytic)	(Grønli et al. 2005) (Schnoor et al. 2011)
18:2ω9c	Fungal-feeding nematodes	(Chen et al. 2001) (Ruess et al. 2004)
18:2ω7c	AM fungi	(Olsson 1999) (Kaiser et al. 2015)
18:0	Eukaryotes and bacteria	(Bååth 2003)
Cy19:0		
20:4ω6c		

Table II.7 Standard Substances for PLFA measurement

Substance	Predominant origin	Reference
14:0	Bacteria (general)	(Kaiser et al. 2015)
i15:0	Gram-positive bacteria	(O'Leary & Wilkinson 1988) (Klamer & Bååth 1998) (Zelles 1997; Zelles 1999)
a15:0	Gram-positive bacteria	(O'Leary & Wilkinson 1988) (Klamer & Bååth 1998) (Zelles 1997; Zelles 1999)
15:0	Bacteria (general)	(Frostegård & Baath 1996) (Bååth & Anderson 2003)
i16:0	Gram-positive bacteria	(O'Leary & Wilkinson 1988) (Klamer & Bååth 1998) (Zelles 1997; Zelles 1999)
16:1ω7c	Gram-negative bacteria	(Wilkinson 1988) (Zelles 1999)
16:1ω5c	AM fungi Bacteria Gram-negative bacteria	(Olsson et al. 1995; Olsson 1999) (Frostegård et al. 2011) (Grayston et al. 2001)
10Me16:0	Actinomycetes	(Kroppenstedt 1992; Kroppenstedt 1985)
i17:0	Gram-positive bacteria	(O'Leary & Wilkinson 1988) (Klamer & Bååth 1998) (Zelles 1997; Zelles 1999)
a17:0	Gram-positive bacteria	(O'Leary & Wilkinson 1988) (Klamer & Bååth 1998) (Zelles 1997; Zelles 1999)
17:0	Bacteria (general)	(Frostegård & Baath 1996) (Bååth & Anderson 2003)
18:2ω6,9	Fungi (saprophytic)	(Frostegård & Baath 1996) (Olsson & Wallander 1998) (Bååth & Anderson 2003) (Frostegård et al. 2011)
18:2ω9c	Fungi (saprophytic) Bacteria	(Sakamoto et al. 2004) (Frostegård et al. 2011) (Schoug et al. 2008)
18:2ω7c	AM fungi Gram-negative bacteria	(Olsson 1999) (Wilkinson 1988) (Zelles 1999)
18:0	Stress indicator	(Moore-Kucera & Dick 2008)
Cy19:0	Gram-negative bacteria	(Vestal & White 1989)
20:4ω6c	Protozoan	(Ringelberg et al. 1997)

Table II.8 Composition of multistandard for the determination of fatty acid concentrations

Single standard	Concentration in Multistandard (µg/µl)
Straight chain saturated fatty acids	
14:0	0.0300
15:0	0.0299
17:0	0.0299
18:0	0.0299
Cyclopropyl branched fatty acids	
Cy19:0	0.0300
10-Methyl branched saturated fatty acids	
10Me16:0	0.0300
Anteiso branched saturated fatty acids	
a15:0	0.0300
a17:0	0.0302
Iso branched saturated fatty acids	
i15:0	0.0284
i16:0	0.0300
i17:0	0.0300
Monosaturated straight chain fatty acids	
16:1ω5c	0.0300
16:1ω7c	0.0300
18:1ω7c	0.0300
18:1ω9c	0.0291
Polyunsaturated straight chain fatty acids	
18:2ω6,9c	0.0300
18:3ω3,6,9c	0.0300
20:4ω6,9,12,15c	0.0300

2.1.7 Software

Table II.9 Software

Software	Characteristics	Web address
ABI Prism®7300 SDS Software 1.4	Software to run the ABI Prism®7300 Sequence Detection System and for data evaluation	https://www.thermofisher.com/de/de/home/technical-resources/software-downloads/abi-prism-7000-sequence-detection-system.html
ionOS 3.0	Software for stable isotope processing	http://www.isoprime.co.uk/products/software/ionos.html
NIS-Elements AR 4.40.00 software	Software for processing fluorescence microscopy images	https://www.nikoninstruments.com/en_DE/Products/Software/NIS-Elements-Advanced-Research
R 3.3.2	Software for statistical analysis	https://www.r-project.org
NCBI Homepage	Internet databank for sequence analysis and literature research	http://ncbi.nlm.nih.gov/

2.1.8 Plant material

Solanum lycorpersicum, cultivar Moneymaker (Volmary, Münster, Germany)

Medicago truncatula, Gaertn. Jemalong, line A17 (provided by Prof. Dr. Helge Küster, Leibniz Universität Hannover, Institute of plant genetics)

2.2 Methods

2.2.1 Experimental Design

2.2.1.1 Experimental Design of the One Arm Experiment

The set-up for the One Arm Experiment consists of two compartments connected via two holes of a 2 cm diameter. To build the compartments, nalgene bottles were chosen, due to their inertness and stability. One compartment is open on top and contains the mycorrhized plant. It is further called the root compartment (RC), because roots and hyphae are growing in this compartment. The second compartment can only be reached by the hyphae and is therefore called the hyphal compartment (HC). The HC can contain different sources of phosphate only the hyphae should be able to reach. The holes between the compartments are covered with a 20 µm mesh to prevent the roots from growing in the hyphal compartment and a PTFE membrane to prevent diffusion of the nutrient to the root compartment (Mäder et al. 1993; Frey et al. 1998; Mäder et al. 2000). The compartment only accessible for the fungus is an intact Nalgene bottle with a screw lid, which allows the connection of a CO_2 gas analyzer, the EGM-4. A lid with two tubes attached to it can be screwed on the fungal bottle, and the tubes can be connected to the EGM-4. Then the CO_2 level and its changes can be measured and the CO_2 flux is generated by the EGM-4. Figure II.1 shows a sketch of the design.

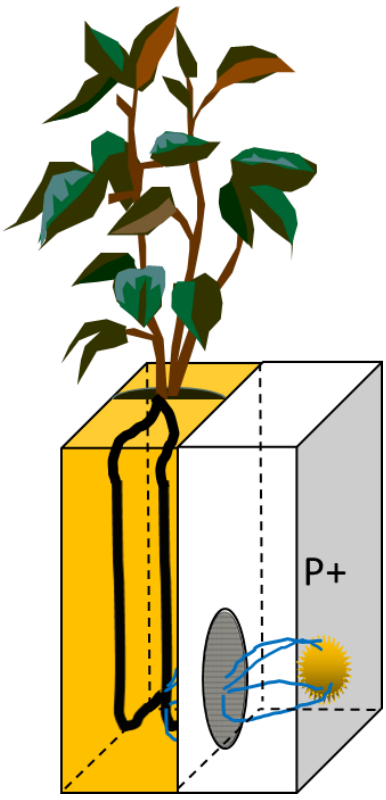

Figure II.1: Sketch of the One Arm experimental design. Roots (black) and mycelium (blue) grow together in the root compartment (RC, yellow). Only the hyphae can grow into the second compartment (HC, white). The phosphate source is given in the HC, so only the hyphae have access to it.

The experiment was carried out with 225 one arm mesocosms. In each RC, a six weeks old mycorrhized plant was transplanted. For each plant, the stem width at the bottom and at the top, the number of leaves and the height of the plant were measured. Four different nutrient sources were used in the hyphal compartment: biotite (15 g), apatite (3 g), a 2.15 mM phosphate solution, and a 0.36 mM phytate solution (60 ml of each). The two solutions contained an equal amount of phosphorus. For each of these treatments, 35 one arm mesocosms were prepared. The HCs were filled with autoclaved quartz sand washed with 10% HCl and with the respective nutrient source. In the compartments with biotite and apatite, 60 ml of deionized and autoclaved water was added additionally. 25 compartments were filled only with quartz sand and 60 ml of deionized and autoclaved water and served as a control. Another 25 closed bottles (screw top) with 200 ml quartz sand, which had no connection to a second bottle, were also included as an additional control for CO_2 measurements. There were five of these control bottles for each nutrient source. Controls helped to check the background breathing which may occur due to a contamination with bacteria. The screw tops of all the HCs were then covered with a black net fabric, which was hold in place by a rubber band. This allowed gas exchange with the surrounding air and avoided a CO_2 accumulation in the substrate, while simultaneously lowering the light intensity inside of the compartment. The plants were irrigated with a wick system with deionized water, and they were fertilized three times a week with a Long Ashton nutrient solution containing no phosphate. The plants with biotite in the hyphal compartment, as well as ten of the control plants, were fertilized with a Long Ashton nutrient solution without potassium.

In the first week, the breathing rate of each hyphal compartment was measured once for 3 minutes with the EGM-4. Each day, 7 bottles of each nutrient source as well as five of the control bottles were measured. The same schedule applied for the second week, but the measurement time was shortened to 90 s. This schedule was kept during the whole experiment, which lasted for 24 weeks.

There were six harvesting points, which were 35, 56, 85, 112, 142, and 165 days after the transplantation of the plants. At each harvesting point, five plants from each treatment and three plants from the control group were harvested. At the last time point, all the plants still left in the experiment were harvested, which were 10 plants per treatment, except for biotite, where only 5 plants were left due to starvation of 5 plants during the experiment. The whole experiment was planned, designed and carried out in close collaboration with Alberto Andrino and the work in measuring the CO_2 flow and harvesting the mesocosms was split equally. Alberto Andrino calculated the necessary amounts of nutrients in the fertilization and of the phosphate sources for each treatment.

2.2.1.2 First Experimental Design of the Split Root System

A split root set-up was designed where the roots were divided into two identical compartments. Figure II.2 shows a sketch of the design. Autoclaved quartz sand was used as substrate in all treatments, because of its naturally low phosphate and carbon content. Both roots and hyphae had access to the phosphate source present in one or both of the compartments. The two compartments were irrigated and fertilized individually with a wick system. Plants were fertilized with a modified Long Ashton solution containing either 0.32 mM phosphate (P+) or no phosphate (P-) (Table II.4). The phosphate concentration on the P+ side was chosen based on previous experiments to maintain a stable mycorrhization and is supplying a minimal amount of phosphate. All systems were watered in addition with deionized water as needed.

Two experiments with *Solanum lycopersicum* were carried out in this design. In the first experiment, a P+ situation on one side of the split root system was compared to a P- situation on the other side. The experiment was carried out with 25 plants in the P+/P- situation. As a control, 10 systems were included which received the P+ solution on both sides. Furthermore, 10 systems were set up with unmycorrhized plants as an additional control.

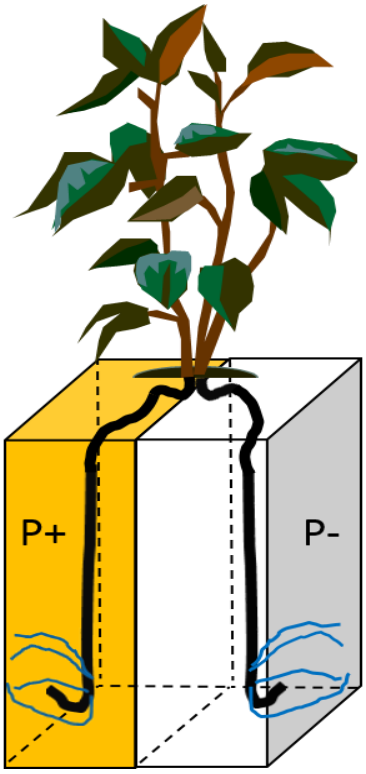

Figure II.2: Sketch of design 1 for the split root system. Roots (black) and mycelium (blue) grow together in both split root compartments. The compartments can be fertilized with different P sources, either with a wick system and different nutrient solutions or by adding the P source directly into the compartment. Each side is filled with autoclaved quartz sand as a substrate.

In the second experiment, apatite was introduced as a second phosphate source. There were five different treatments and six replicates for each treatment. The treatments were (i) P+ solution on both sides (PP), (ii) P+ on one side and P- on the other side (NP), (iii) apatite on both sides (AA), (iv) apatite on one side and P- on the other side (AP), and (v) apatite on one side and P+ on the other side (AN). The P solutions were the same as in the first experiment. The apatite was given as 6 g of small grains (3-5 mm) per compartment.

The second experiment was repeated with *M. truncatula*. For *M. truncatula*, five replicates for each treatment were used. Instead of a Long Ashton solution, a modified Hoagland solution was used (Table II.4), and instead of 6 g of apatite, only 3 g of apatite were used. Stefanie Wegener assisted me with the breeding of the *M. truncatula* plants and provided me with the necessary information about plant inoculation and fertilization.

2.2.1.3 Second Experimental Design of the Split Root System

A second split root system was designed for the experiments with *M. truncatula*. Figure II.3 shows a sketch of the design. The roots are also divided into two identical compartments, called the root compartments (RC). These compartments (RC) are each connected via a 2 cm hole to a second compartment, the hyphal compartment (HC). The hole is covered with a PTFE membrane, which prevents diffusion of nutrients between the compartments and which only the hyphae are able to cross (Mäder et al. 1993; Frey et al. 1998; Mäder et al. 2000). The HCs contain the phosphate source, so that only the fungus has access to it. They can be closed via a screw lid, which allows access to the compartment throughout the experiment, without disturbing the system. The two RCs of each set-up were irrigated and fertilized individually with a wick system. Both root compartments were fertilized with a modified Hoagland solution containing no phosphate and irrigated additionally with deionized water when necessary.

There were five different treatments and five replicates for each treatment. The treatments were (i) P+ solution on both sides (PP), (ii) P+ on one side and P- on the other side (NP), (iii) apatite on both sides (AA), (iv) apatite on one side and P- on the other side (AN), and (v) apatite on one side and P+ on the other side (AP). The P+ HCs were receiving 20 ml of a 1000 mM $KHPO_2$ solution, the P- HCs received 20 ml of deionized water instead. One week before harvesting, each compartment containing $KHPO_2$ received five additional ml of the solution, the P- HCs received 5 ml of deionized water. The HCs with apatite as a phosphate source received 3 g of apatite in small grains (3-5 mm) in the HC at the beginning of the experiment, equally mixed with autoclaved and 10% HCl washed

quartz sand. They also received 20 ml of deionized water. The experiment was carried out two times. At the second time, the plants were pulse labeled with ^{13}C for 2 hours 3 days before harvesting.

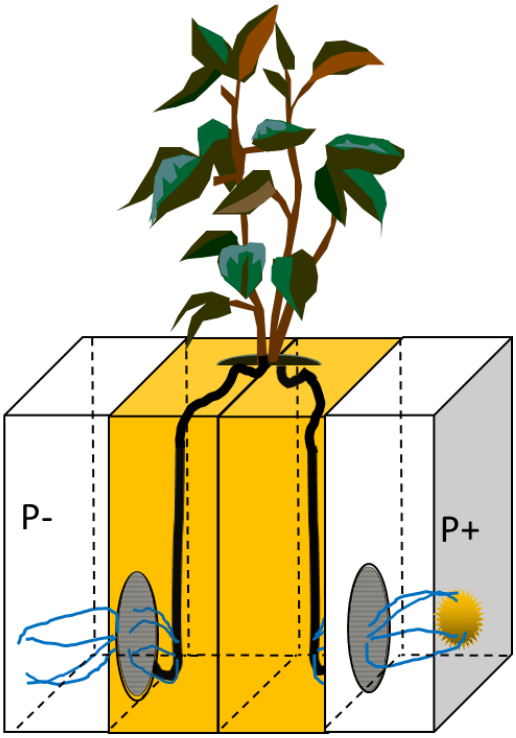

Figure II.3: Sketch of design 2 for the split root system. Roots (black) and mycelium (blue) grow together in the first two compartments (RC, yellow). Only the hyphae can grow into the second compartment (HC, white). The phosphate source is given into the HC, so only hyphae have access to it. The RCs can be fertilized and irrigated individually with a wick system. Each side is filled with autoclaved quartz sand as a substrate.

2.2.2 Plant Breeding

2.2.2.1 Production of *Rhizophagus irregularis* Inoculum

Seeds of *Sorghum bicolor* were planted in flower boxes (80 x 17.5 x 14.5) in a 70/30 mixture of autoclaved percolite and quartz sand. Seeds were inoculated with 30 g of *R. irregularis* inoculum (Table II.1) and the inoculum was covered under 1 cm of autoclaved quartz sand. The plants were kept in the green house at 24 °C with 16 h of additional light for 10 weeks and watered with tap water 3 times a week. Mycorrhization was checked every third week. The fully mycorrhized plants were dried directly in the flower boxes for 3 weeks. The shoot material was cut off, and the inoculated roots were cleared of percolite and quartz sand. The roots were kept at 4 °C in the dark until further usage and were only used in experiments with *Solanum lycopersicum*. The production of the *R. irregularis* inoculum was developed and carried out by Alberto Andrino.

2.2.2.2 Seed Sterilization

Seeds of *Solanum lycopersicum* were sterilized with 2% sodium hypochlorite for 6 min and washed with water for three times. Seeds were then put on a filter paper for three days at 25 °C degrees in the dark for germination.

Seeds of *Medigaco truncatula* were incubated in 98% sulfuric acid for 10 min and washed three times with deionized water to get rid of sulfuric acid leftovers. Seeds were then incubated in 20 % sodium hypochloride for 10 min and again washed with deionized water for three times. Afterwards, seeds were incubated in sterilized water for 20 min for swelling. They were then put on a plate with water agar (phyto agar 7.5 g/l), which were wrapped in foil to keep the light out. The plates were kept in the dark at 4 °C for two days for vernalisation, and then transferred to 24 °C in the dark for two more days for germination. The germinated seeds were kept at 23 °C and 350 µmol*$m^{-2}s^{-1}$ light intensity for 10 days.

2.2.2.3 Inoculation and Plant Growth

The germinated *Solanum lycopersicum* seeds were planted in multi pot plates (4 cm in diameter, 7 cm height per pot; one seedling per pot) in autoclaved quartz sand. They were inoculated with 3 g of *Rhizophagus irregularis* inoculum (self-made, see section 2.2.2.1) directly into the potting hole. Seedlings were grown for 6 weeks in the greenhouse at 24°C and fertilized 3 times a week with 5 ml of a modified Long Ashton Solution (Table II.4) containing 0.32 mM phosphate. The amount of phosphate was chosen based on previous experiments to ensure maximal mycorrhization. All plants were additionally watered with tap water each morning as needed.

The germinated *Medicago truncatula* seedlings were potted in 13 cm diameter pots with a 50/50 mixture of sterile quartz sand and vermiculite. Each plant was inoculated with 3 g of *R. irregularis* inoculum (Table II.1) directly into the potting hole. Seedlings were then grown for 6 weeks in a phytochamber (Table II.2) at 23 °C during the day and 18 °C at night. The light intensity was 350 µmol*$m^{-2}s^{-1}$ for 16 hours per day. Humidity was around 65%. The program is based on a publication by Sulieman et al. (2013). The seedlings were watered with demineralized water as needed until three weeks after potting. They were then supplied with 50 ml of a 0.5x Hoagland solution (Table II.4) containing 1 mM phosphate once a week and additionally watered with demineralized water when necessary.

2.2.3 Plant Treatment in the Experiments

2.2.3.1 Treatment in the One Arm Experiment

After 6 weeks, the mycorrhized plants were transplanted into the bigger pot of the One Arm Design. Autoclaved quartz sand was used as a substrate. The autoclaved quartz sand in the outer chamber was additionally washed with 10% HCl. The plants were kept in the greenhouse at 24 °C and with 16 h of additional light. They were fertilized 3 times a week with 20 ml of a modified Long Ashton solution (Table II.4) containing either no phosphate (P-) or no potassium (K-). They were watered additionally with deionized water when necessary.

2.2.3.2 Treatment in the Split Root Experiments

After 6 weeks of growth, the plants were dug out gently, and the roots were washed with deionized water. The roots were distributed equally between the two split root compartments. Autoclaved quartz sand was used as substrate in all treatments. Each side was inoculated again with 3 g of *R. irregularis* (section 2.2.2.1) inoculum.

The *Solanum lycopersicum* plants were grown in the split root system for 6 weeks in the greenhouse at 24 °C and with 16 h of additional light. They were fertilized 3 times a week with 10 ml of a modified Long Ashton solution (Table II.4) per compartment containing either 0.32 mM phosphate (P+) or no phosphate (P-). The two compartments were irrigated and fertilized individually with a wick system. All systems were watered in addition with deionized water when necessary.

The *Medicago truncatula* plants were grown in the split root system for 6 weeks in the phytochamber (Table II.2), after a program published by Sulieman et al. (2013). The temperature was 23 °C during the day and 18 °C at night, with a light period of 16 hours and a light intensity of 350 µmol*$m^{-2}s^{-1}$. The humidity was around 70% at all times. In design 1, the plants were fertilized two times a week with 50 ml per compartment of a 0.5x Hoagland solution (Table II.4) containing either 0.3 mM phosphate (P+) for the P+ compartments or no phosphate (P-) for the P- and the apatite compartments. In design 2, they received 50 ml of the P+ solution per compartment in the first week for two times. From the second week on, they were fertilized two times a week with 50 ml of the P- solution per compartment. All plants were watered additionally with deionized water when necessary.

2.2.4 Physiological measurements

2.2.4.1 RNA Extraction

For the RNA extraction, the root material was frozen in liquid nitrogen immediately after the harvest of the plant. The frozen material was ground in liquid nitrogen and stored at -80 °C. Total RNA was extracted from 80 mg of ground root material with the NucleoSpin RNA Plant Kit (Table II.1) according to the manufacturer's instructions. 350 µl of buffer RA1 with 10 µl of a 500 mM DTT solution was used for *S. lycopersicum*. For *M. truncatula*, 600 µl of buffer RA1 with 15 µl of a 500 mM DTT solution was used. RNA was eluated with 60 µl of H_2O. RNA quality was assessed by agarose gel electrophoresis (Sambrook et al. 1989), and quantification was performed using the BioTek Synergy MX (Table II.2).

2.2.4.2 cDNA Synthesis

Total RNA (100 ng) was treated again with DNase (Table II.1) for 30 min at 37 °C and 15 min at 70 °C to destroy the DNase. The RNA was then used for cDNA synthesis with an oligo $d(T)_{15}$ primer (Table II.3) in a 20 µl reaction volume following the manufacturer's protocol of the MMLV-reverse transcriptase (Table II.1). 1 µl of the primer was added and the sample was incubated at 70 °C for 5 min to allow primer annealing. The sample was then cooled on ice for 10 min. 2 µl dNTPs, 4 µl buffer, 0.5 µl enzyme and 0.5 µl H_2O were added to each sample. The samples were incubated at 37 °C for 60 min and then 70 °C for 10 min to destroy the reverse transcriptase.

2.2.4.3 Quantitative PCR

The cDNA was diluted 1:10 in H_2O, and 2 µl were used for qPCR in a 20 µl volume using 10 µl absolute SYBR green mix (Table II.1), 7.2 µl H_2O and 0.4 µl of each gene specific primers (10 µM) (Table II.3). The qPCR was carried out on a qPCR cycler (Table II.2). The reaction was started at 95 °C for 15 min, followed by 40 cycles at 95 °C for 10 s, 60°C for 30 s and 72°C for 30s. Dissociation curves were generated at the end of the PCR cycles from 70 to 95 °C to test for unspecific amplicons. Serial dilutions of the cDNA template allowed for estimating the primer pair efficiency. RNA samples were checked for genomic DNA contamination by performing a control PCR on RNA not reverse-transcribed. No-template controls were used for each primer pair. All data were analyzed using the SDS 2.3 software (Applied Biosystems) and a standard curve was calculated with the dilution series for each run and primer pair with the software. Primer pairs for the phosphate transporters *LePT4* and *LePT3*, the sugar transporters *SlSUT1* and *SlSUT2* (Boldt et al. 2011) and the fungal transporter

genes *GiPT* and *GiMST2* (Helber et al. 2011) were used. The housekeeping gene *LeTefα*, encoding the translation elongation factor EF-1α, was used as a normalizer (Schaarschmidt et al. 2006; Boldt et al. 2011) for each tomato gene. For *M. truncatula*, *MtTefα*, encoding the same gene, was used as a normalizer, respectively (Hohnjec et al. 2003). The fungal gene *GiTefα,* encoding the translation elongation factor EF-1α, and a region from the fungal 5.8S gene (*GiRNS*) were used as a normalizer for the fungal transporter genes and to confirm the mycorrhizal rate obtained by staining procedures and microscopic analysis (Benedetto et al. 2005; Olsson et al. 2006; Helber et al. 2011; Gomez et al. 2009).

2.2.4.5 Measurement of Nutrient and Phosphate Status of Leaves and Roots

Root material was ground in liquid nitrogen and stored at -80 °C. To determine the inorganic phosphate concentration of the roots, a photometric test was performed according to an adapted protocol of Taussky & Shorr (1953). 800 µl of 3% perchloric acid was added to 100 mg of ground root material. The material was shaken for 20 min and centrifuged at 14 000 x g for 5 min. 120 µl of the supernatant was then transferred to a 96 well plate for photometric tests. 80 µl of the reaction solution (10 ml of 10% (w/v) $(NH_4)_6Mo_7O_{24}$ in 10 M H_2SO_4, 0.18 M $FeSO_4$ in H_2O) were added to each well. A dilution series with KH_2PO_4 was used as a standard. The photometric measurement was done with the BioTek Synergy MX (Table II.2) at 750 nm absorption.

Stems and leaves of the plants were dried and milled with a ball mill (MM400, Table II.2). 80-100 mg of the powder was burned in a muffler oven (Table II.2) at 480 °C over night. The ashes were taken up with 1 ml of 20% HNO_3 and 9 ml H_2O. The liquid was filtered with a 2.5 µm cellulose filter (110 mm, Whatman, Maidstone, UK) and the nutrient status of P, K, Mg, Ca, B, Mn, and Fe was then measured with the ICP-MS (Table II.2). The preparation of the samples from the One Arm experiment has been done by Alberto Andrino.

2.2.4.6 Determination of Mycorrhizal Colonization

The degree of mycorrhization was determined by ink staining for *Solanum lycopersicum* and AlexaFluor® (Table II.1) staining for *Medicago truncatula* followed by microscopy using the grid line intersection method (Giovannetti & Mosse 1980; Brundrett et al. 1996) or the magnified intersection method of McGonigle et al. (1990).

For the ink staining, fresh root samples were taken and cooked in 10% KOH at 90 °C for 25 min. Root samples were washed 3 times with deionized water. Afterwards, the root samples are kept in

0.1 M HCl for 5 min. The HCl was removed and the ink was added instead. Root samples were stained for 15 min at room temperature. The ink was removed and the root samples were kept in Glycerol until microscopy analysis. The mycorrhization was analyzed using the grid line intersection method (Giovannetti & Mosse 1980; Brundrett et al. 1996). The staining of the roots as well as the analyzation of the mycorrhization for the *Solanum lycopersicum* plants was done by Alberto Andrino.

For the AlexaFluor® (Table II.1) staining, a stock solution was prepared containing 20 µg/ml AlexaFluor® in 1 x PBS buffer (Table II.4). The stock solution was stored at -20 °C. Before each staining, a 1:50 dilution of the stock solution with 1 x PBS buffer was made. The fresh root pieces were cooked in 10 % KOH at 90 °C for 10 min and rinsed with deionized water 3 times. Then, each root sample was covered with the diluted AlexaFluor® solution and kept over night (at least 12 h) at room temperature in the dark. The next day, the staining solution was washed away with water 3 times and the roots were kept at 4 °C in 1 x PBS buffer in the dark until microscopy analysis. With the stained roots, the mycorrhizal colonization was determined using the magnified intersection method of McGonigle et al. (1990). Fluorescence microscopy was performed with the Nikon Eclipse Ti fluorescence microscope (Table II.2). Images were taken with a Nikon Plan Apo, 10x/0.45 objective. The AlexaFluor® fluorescence was analyzed by excitation at 480/20 nm. Image processing was done with the NIS-Elements AR 4.40.00 software (Table II.9).

The mycorrhizal rate was also determined via qPCR. Therefore, the root samples were ground in liquid nitrogen and RNA was extracted as described above. cDNA was synthesized and a qPCR was performed as described in the respective chapters. Primer pairs for the fungal gene *GiTefα* encoding the translation elongation factor EF-1α (Liu et al. 2007; Corradi et al. 2004), and for the 5.8S gene (*GiRNS*) (Isayenkov et al. 2004) were used in the qPCR. A standard curve was calculated using the SDS 2.3 software (Applied Biosystems), based on a dilution series for each run and primer pair accordingly. Each value was normalized with the plant housekeeping gene *LeTefα*, encoding the translation elongation factor EF-1α.

2.2.4.7 Carbon and Nitrogen Analysis

To measure the carbon and nitrogen content of the plants, stems and leaves of *Solanum lycopersicum* were dried at 105 °C in a drying oven (Table II.2). The dried samples were milled with a ball mill (MM400, Table II.2) to a fine powder. 5-10 mg of each sample was weighted in tin capsules. The capsules were closed and measured with the CN analyzer (Vario EL III, Table II.2). For the One Arm experiment, the preparation of the samples was done by Alberto Andrino.

For *M. truncatula*, roots and leaves were ground in liquid nitrogen directly after harvesting. After the extraction of samples for RNA and PLFA analysis, the ground samples were dried at 55 °C (Table II.2). The dried samples were milled with a ball mill (MM400, Table II.2) to a fine powder. 5-10 mg of each plant sample was weighted in tin capsules. The measurement was done with the CN analyzer (Vario EL III, Table II.2). The calibration was done with acetanilide.

2.2.5 ^{13}C Tracer Experiment

2.2.5.1 $^{13}CO_2$ Pulse Labeling

Three days before the harvesting of the *Medicago truncatula* plants in design 2, a $^{13}CO_2$ pulse labeling was done. The labeling was performed in the phytochamber (Table II.2) with a light intensity of 350 µmol $m^{-2}s^{-1}$ and a temperature of 23 °C. All plants were transferred into an airtight transparent foil box (45 cm x 60 cm x 130 cm). 1 g of ^{13}C sodium carbonate (Table II.1) was placed in the box and covered with 10 ml of 1 M sulfuric acid. Three fans inside the box were used for ventilation, and to allow an even distribution of $^{13}CO_2$ in the box. Labeling was done for 2 hours. Afterwards, the foilbox was opened outside of the phytochamber to avoid ^{13}C contamination of the chamber, and plants were transferred back into the phytochamber.

2.2.5.2 Measurement of ^{13}C isotopy in plant samples

M. truncatula roots and leaves were ground in liquid nitrogen and the ground samples were dried at 55 °C (Table II.2). The dried samples were milled with a ball mill (MM200, Table II.2) to a fine powder. 5-10 mg of each plant sample and ~120 mg of each substrate sample was weighted in tin capsules. The measurement was done with the elementar analyzer (EA) (Vario isotope cube, Table II.2) coupled with an isotopic ratio mass spectrometer (IRMS) (Isoprime, Table II.2) for carbon content and stable isotope composition ($\delta^{13}C$).

In the EA analyzer, the tin capsules with the samples are burned in the oxidation tube filled with cerium oxide (CeO_2) at 1080 °C in the presence of an oxygen atmosphere. The burned sample is lead through the reduction tube, filled with elemental copper (CU), where the nitric oxides are reduced to N_2 at 600 °C. Now, the CO_2 is diluted with helium (he). The diluter can be disconnected, if CO_2 would be too low to reach a peak intensity of 1 nA. The measurement of carbon and nitrogen concentrations is done with a thermal conductivity detector. It is calibrated with acetanilide for plant samples and high organic sediment (HOS) for soil samples. For labeled samples, the ^{13}C isotopy

measurement with the IRMS is following. The calibration is performed using one pulse of a CO_2 reference gas (Linde AG, Hannover, Germany, purity 99.99995%).

To calibrate the measurements, different standard substances are also weighted in and measured with the samples. A list of these standards can be found in Table II.5. For ^{13}C, caffeine, cellulose and $CaCO_3$ are used as reference material. For the referencing, a linear calibration with these standards is done. Caffeine and cellulose are both IAEA standards. The $CaCO_3$ is an in-house standard, which was measured 40 times and referenced with the IAEA standards prior to its use. Two additional in-house standards, quartz sand and needle litter, were used as a further control to ensure a consistent measurement and to detect possible drifts throughout the measuring process.

The ^{13}C values were corrected with the ^{13}C values of plants not labeled, but grown under the same conditions as the labeled plants. In addition, the isotopic composition was calculated with the Vienna Pee Dee Belemnite (VPDB) standard:

$$^{13}C(atom\,\%) = \frac{(100 * 0{,}0111803 * (corrected \frac{^{13}C}{1000+1}))}{(1 + 0{,}0111803 * \left(corrected \frac{^{13}C}{1000+1}\right))}$$

2.2.6 Analysis of Fungal Fatty Acids

The phospholipid fatty acids (PLFAs) and neutral lipid fatty acids (NLFA) analysis is based on a modified protocol from Bligh & Dyer (1959) and White et al. (1979).

2.2.6.1 Extraction of Fatty Acids

For the extraction, ~100 mg of frozen and ground root material was used. 18 ml of a Bligh & Dyer solution was added to each sample in addition with 25 µl of the internal standards for PLFA (IS1, 19:0, 1 g/l) and NLFA (IS3, 12:0, 1 g/l). The samples were shaken in a horizontal shaker at 300 rpm for 15 min. They were put in an ultrasonic bath for 15 min and shook again on the horizontal shaker for another 15 min. They were then centrifuged at 3000 x g for 12 min at 7 °C (Cryofuge 6000, Table II.2) and the supernatant was transferred into 250 ml separating funnels. 20 ml of Bligh & Dyer solution were added again to each sample and they were shaken on the horizontal shaker at 300 rpm for

15 min. The samples were centrifuged again at 3000 x g for 12 min at 7 °C (Cryofuge 6000, Table II.2) and the supernatant was transferred to the rest of the sample in the separating funnels. 20 ml of chloroform and 20 ml of citrate buffer was added to each sample to establish a two-phase solution. The separating funnels were shaken for 15 min at 250 rpm on a horizontal shaker. Afterwards, they were kept upright for 1-2 h to establish a clear phase boundary. The lower phase was collected in 50 ml bottom copped flasks and put aside. Additional 20 ml of chloroform were added to each separating funnel and the funnels were shaken again for 15 min on the horizontal shaker. They were kept upright over night to establish the phase boundary.

The lower phase in the separating funnels was collected again with the rest of the sample in the copped bottom flasks. The sample volume was than reduced to ~1 ml with the use of rotation evaporators (Table II.2). Glass columns were prepared with a filter, followed by 1.5 cm of activated silica gel in chloroform (high-purity grade, pore size 60 A), capped with a second filter, to separate the different lipid phases in the chloroform samples. Each sample was given on one of the columns, and the copped bottom flasks were rinsed three times with ~ 0.5 ml of chloroform, which was also given on the columns. Additionally, 5 ml of chloroform was added to each column. With the chloroform, the NLFAs were isolated from the samples. The chloroform was eluted slowly with a velocity of two drops per second into new 50 ml copped bottom flasks. Afterwards, the columns were filled with 20 ml of acetone. The acetone elutes the glycolipids, which are not needed for further analysis. Therefore, the elution of acetone, again with a velocity of 2 drops per second, can be discarded. Thereafter, 40 ml of methanol are added in two steps to the columns to elute the NLFAs. The methanol is collected in 50 ml copped bottom flasks and the elution is again carried out with a velocity of 2 drops per second. The chloroform and methanol samples in the copped bottom flasks were reduced with the rotary evaporator to a volume of ~0.5 ml and the samples were transferred to reactivials. The copped bottom flasks were rinsed 3 times with either chloroform or methanol in the process. The samples were dried under a gentle nitrogen stream and kept at -20 °C until derivatization.

The dried and frozen samples need to be redissolved for the derivatization. Therefore, 0.5 ml of a 0.5 M NaOH in MeOH solution was added to each sample. They were then put in an ultrasonification bath for 10 min and mixed with a vortex at full speed for 5 min. Afterwards, the samples were put in a heated derivatization block at 100 °C for 10 min. 0.75 ml of a 12.5 M BF_3 solution (14% in methanol) was added after the samples had cooled down to room temperature. The samples were put again on the derivatization block at 80 °C for 15 min and then cooled down to room temperature. 0.5 ml of a saturated NaOH solution was added to reduce the BF_3 and thereby decrease the toxicity of the component. Then, 1 ml of hexane was added to each sample and they were vortexed for 3 min at full speed. A short while after the shaking, two phases occured with a clear phase boundary. The apolar

upper phase was separated with a glass pipette and transferred to a new reactivial. The hexane extraction was repeated two more times, and the extract was dried completely under a gentle nitrogen stream. 185 µl of toluene was added to redissolve each sample. In addition, 15 µl of the second internal standard for fatty acid methyl ester (FAME) (IS 2, 13:0, 1g/l) was added to each sample. The samples were put in the ultrasonification bath for 10 min and then transferred to GC autosampler vials and stored at -20 °C until measurement.

For each set of samples, a standard sequence was prepared. A multistandard consisting of 17 substances in a concentration of 1 g/l per substance in MeOH was prepared in advance. For the detailed composition, see table II.8 in the material section. 7 standards were prepared in reactivials with 25, 50, 150, 300, 600, 800 and 1000 µl of the multistandard. These standards were dried under a gentle nitrogen stream. The dried standards were derivatized together with the PLFA and NLFA samples.

The original PLFA/NLFA extraction protocol has been modified and improved by Alberto Andrino for extraction of PLFA from quartz sand and by Stefanie Wegener for extraction of PLFA and NLFA from *M. truncatula* root samples. The PLFA extraction and analysis of the sand from the HC of the One Arm experiment has been done completely by Alberto Andrino.

2.2.6.2 Measurement of Fatty Acids

The measurement was done with a gas chromatograph (GC) (GC System, 7890A, Table II.2) combined with an stable isotope ratio mass spectrometer (IRMS) (Isoprime, Table II.2) for isotopic analysis. Settings of the GC-IRMS measurement are given in Table II.10. Analysis was performed on the basis of the 17 substances in the multistandard and the 3 internal standards given to the samples.

Table II.10: Settings of the GC-IRMS

Parameter	Setting
Producer	Agilent Technologies 7890A GC-System
Oven temperature	80°C for 1 min, heat rate of 7°C/min up to 180°C, 180°C for 0 min, heat rate of 0.7°C/min up to 19 °C, 190°C for 3.5 min, heat rate of 0.7°C/min up to 209 °C, 209°C for 0 min, heat rate of 50°C/min up to 300°C, 300°C for 5 min
Injector temperature	250°C
Septum purge	3 ml/min
Split modus	Splitless
Splitless time	0.75 min
Column flow	2 ml/min for 61 min, afterwards with 1 ml/min up to 1.5 ml for 6 min
Detector	IRMS
Interface temperature	350°C
Oven temperature (filled with CuO_2)	850°C
H_2O removal	With Nafion tube
Online calibration	With ref. gas CO_2 (Linde AG, Hanover, Germany, purity: 99.99995%)
Referencing	International standards -> pure substances were measured with EA-IRMS and referenced to international standards themselves

2.2.7 Statistical Analysis

For the split root experiments, normalized data were log-transformed and analyzed in linear mixed effects models with treatments as fixed effects and the plant ID as random effect in order to account for the paired measurements from individual plants. Based on the estimated fixed effect parameters, treatment differences were tested using multiple contrasts as described in Hothorn et al. (2008), with Satterthwaite degrees of freedom.

For the One Arm experiment, as well as for the data from the shoot material of the split root experiments, multi-factor analysis of variance (ANOVA) combined with a Tukey's test for P value adjustment was performed. The analysis of the cumulative P gained by the plants per treatment, the ratio of cumulative CO_2 to total C in the HC, and every analysis concerning the PLFAs of the One Arm Experiment have been done by Alberto Andrino.

Statistical analysis was performed in R 3.3.2, using the packages lme4 (version 1.1-12) (Bates et al. 2014) for fitting mixed effect models, lmerTest (version 2.0-33) (Kuznetsova et al. 2016) for computation of Satterthwaite degrees of freedom, and multcomp (version 1.4-6) (Hothorn et al. 2008) for multiple contrast tests.

3. Results of the Split Root Experiment with *Solanum lycopersicum*

3.1 Influence of Partial Phosphaten Starvation in the Split Root Experiment

The aim of the experiment was to determine if the phosphate transporters *LePT4* and *LePT3* are regulated in response to the phosphate availability in the soil in addition to the degree of mycorrhization. Therefore, two sides of a split root system which either received a minimal phosphate solution (P+) or no phosphate at all (P-) (Fig. II.2), were compared. Control plants received a minimal phosphate solution on both sides of the system (P+/P+). Both sides of the split roots were mycorrhized, except for the nonmycorrhized control plants. RNA was extracted from the root system, and transcript levels were determined via qPCR.

3.1.1 Mycorrhization and Phosphate Status of the Plants

Since lack of phosphate might directly influence mycorrhization (Koide & Li, 1990), the degree of mycorrhization was analyzed via ink staining, and with two fungal markers via qPCR (Fig. III.1). The overall degree of mycorrhization was between 40-60% (Fig. III.1, A), with a small amount of arbuscules (6-10%) in all plants (Fig. III.1B). In the nonmycorrhized control plants, no mycorrhization or arbuscules were detected. The P- side had a significantly lower degree of arbuscules compared to the P+ side, and the overall mycorrhization was also about 20% lower on the P- side compared to the P+ side or the P+/P+ plants (~1.5 fold higher on the P+ side). The qPCR with the fungal markers *GiTefα* and *GiRNS* also showed a ~1.8 – 2.3 fold higher abundance in the P+ side of the split roots plants (Fig. III.1D, E).

To test for a functional establishment of the symbiosis, the phosphate status of the plant roots was measured. There were tendencies towards a better phosphate status of the roots of mycorrhized plants compared to unmycorrhized plants, with a significant difference between the P- side of the unmycorrhized plants compared to the P+/P+ plants and the P- side of the mycorrhized plants. The difference between the P+ side and the P- side of the mycorrhized plants was also significant (Fig. III.1C). This shows that the mycorrhization indeed had a positive influence on the phosphate status of the plant, and that a functional symbiosis was developed.

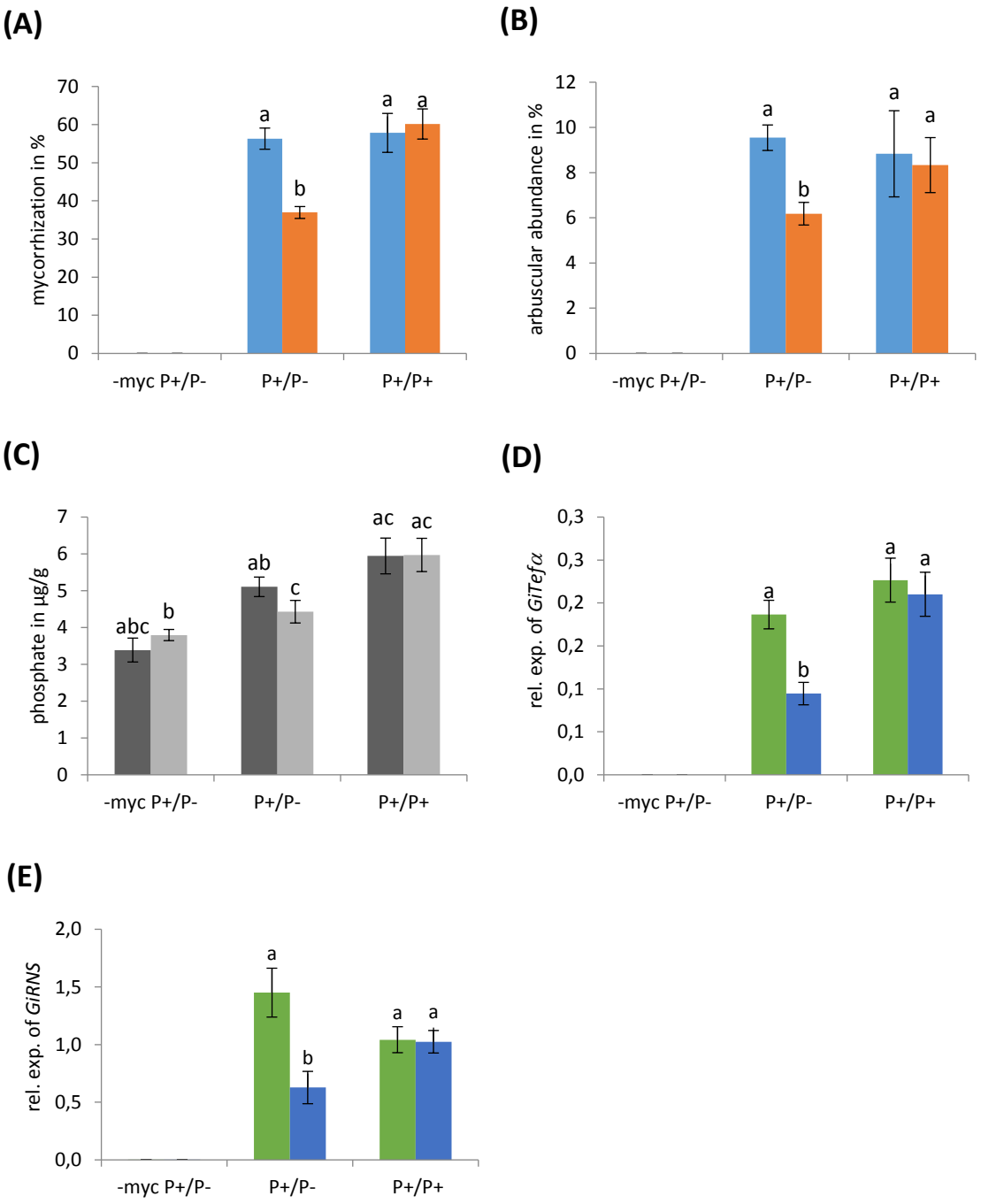

Figure III.1 2: Mycorrhization, arbuscular abundance, relative expression of the fungal marker *GiTefα*, and phosphate content of the plant roots in the first split root experiment. Plants were 12 weeks old with 6 weeks in the split root system. RNA was extracted from the root material. Mycorrhization (A) and arbuscular abundance (B) were obtained using ink staining and microscopy techniques. To determine the root phosphate content (C), a photometric test was performed. Relative *GiTefα* and *GiRNS* transcript levels (D, E) were determined using RT-qPCR analysis with *LeTefα* as a normalizer. -myc: nonmycorrhized plants; P+/P-: plants fertilized with 0.3 mM Pi on the P+ side and no phosphate on the P- side; P+/P+: plants fertilized with 0.3 mM Pi on both sides. Each pair of columns represents a split root system with one column per compartment. Data are given as mean values +/- SE (n=6; P+/P+ n=10; P+/P-, n=25). Different letters indicate significant differences ($p<0.05$).

3.1.2 Phosphate Transporter Gene Expression in Response to Phosphate Starvation

The expression of *LePT4* was ~3.2 fold higher for the roots on the P+ side compared to the P- side (Fig. III.2A). For the plants fertilized on both sides with P, the expression was ~2.4 fold higher in the fertilized roots compared to the P- side of the P+/P- plants. The nonmycorrhized plants showed no expression. The expression pattern of *LePT3* was, with a ~1.5 fold higher expression on the P+ compared to the P- side, similar to the one from *LePT4*, with the exception that it was also expressed in nonmycorrhized plants, though at a reduced rate (~2.3 – 3.3 fold lower) (Fig. III.2B). The differences between the P+ and P- side in the degree of mycorrhization was considerably lower than that for the *LePT4* expression. The data indicates that phosphate availability, in addition to mycorrhization, might also have an influence on *LePT4* expression.

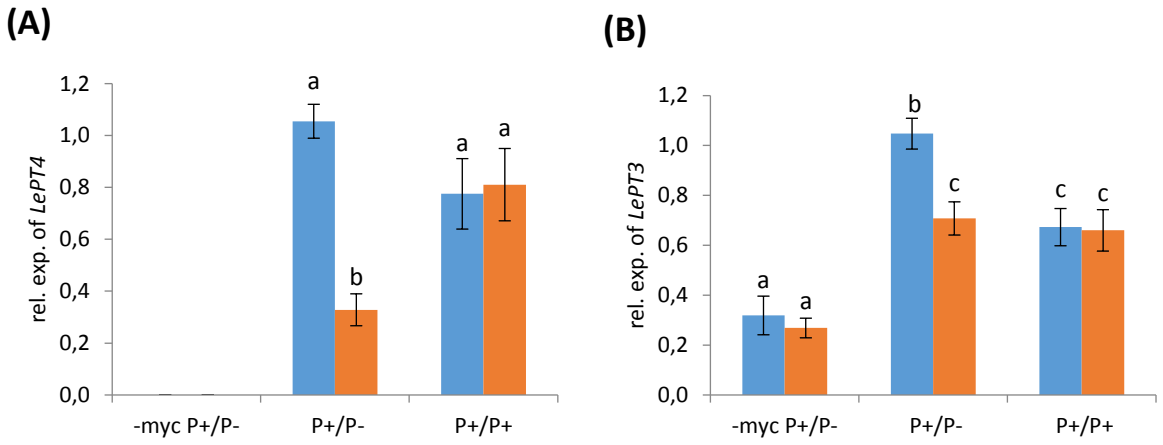

Figure III.2: Relative expression of the phosphate transporters *LePT4* (A) and *LePT3* (B) in the plant roots of the first split root experiment. Plants were 12 weeks old, with 6 weeks in the split root system. RNA was extracted from the root material. Relative *LePT4* and *LePT3* transcript levels were determined using RT-qPCR analysis with *LeTef*α as a normalizer. -myc: nonmycorrhized plants; P+/P-: plants fertilized with 0.3 mM Pi on the P+ side and no phosphate on the P- side; P+/P+: plants fertilized with 0.3 mM Pi on both sides. Each pair of columns represents a split root system with one column per compartment. Data are given as mean values +/- SE (n=6; P+/P+ n=10; P+/P-, n=25). Different letters indicate significant differences ($p<0.05$).

3.1.3 Sugar Transporter Gene Expression in Response to Phosphate Starvation and Mycorrhization

During AM symbiosis, the fungus receives carbon from the plant in exchange for phosphate. Therefore, the expression rate of sugar transporters in root cells might also change in response to the phosphate availability in the symbiosis. Hence, I analyzed if differences in phosphate supply are accompanied with changes in gene expression of the two putative mycorrhiza-induced sugar transporters *SlSUT1* and *SlSUT2* (Boldt et al. 2011). The Expression of both transporters did not change in response to different P availability (Fig. III.3). Instead, *SlSUT1* was significantly higher

expressed in nonmycorrhized plants compared to mycorrhized ones (Fig. III.3A), whereas *SlSUT2* showed no differences in any of the compared situations (Fig. III.3B). This indicates that mycorrhization has a repressing effect on the expression of *SlSUT1*, and that phosphate is not a regulating factor for both sucrose transporters.

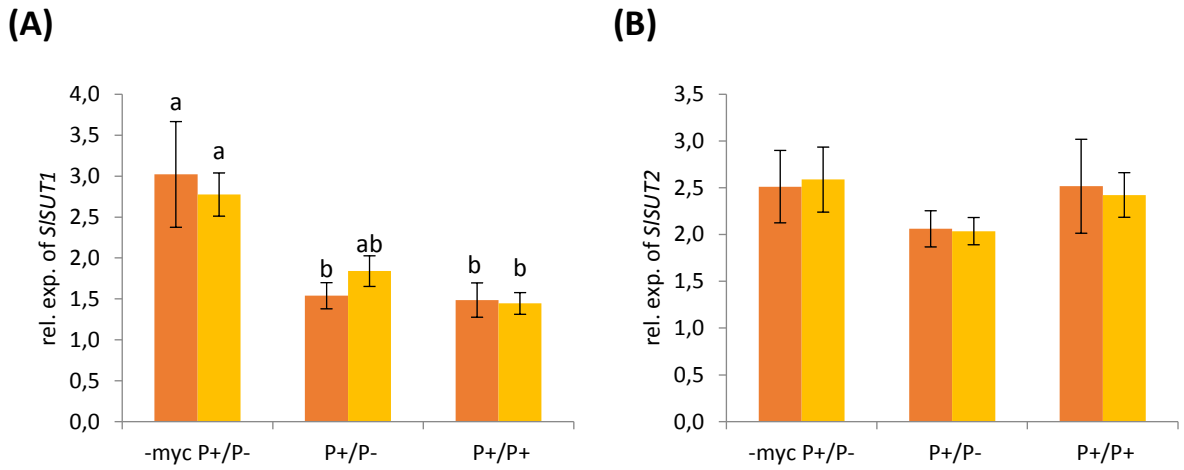

Figure III.3: Relative expression of the sugar transporter *SlSUT1* (A) and *SlSUT2* (B) in the roots of the frirst split root experiment. Plants were 12 weeks old, with 6 weeks in the split root system. RNA was extracted from the root material. Relative *SlSUT1* and *SlSUT2* transcript levels were determined using RT-qPCR analysis with *LeTef*α as a normalizer. -myc: nonmycorrhized plants; P+/P-: plants fertilized with 0.3 mM Pi on the P+ side and no phosphate on the P- side; P+/P+: plants fertilized with 0.3 mM Pi on both sides. Each pair of columns represents a split root system with one column per compartment. Data are given as mean values +/- SE (n=6; P+/P+ n=10; P+/P-, n=25). Different letters indicate significant differences ($p<0.05$).

3.1.4 Fungal Transporter Gene Expression in Response to Phosphate Starvation

To evaluate if the phosphate status of the soil also has an influence on fungal transporters, the expression of the fungal phosphate transporter *GiPT* and the fungal sugar transporters *GiMST2* was analyzed. Expression of both genes was normalized with the fungal marker gene *GiTefα*. The expression of both genes was significantly higher on the P- side compared to the P+ side or compared to the roots of the P+/P+ plants (Fig. III.4), indicating that the soil P status has an effect on the expression of these fungal genes.

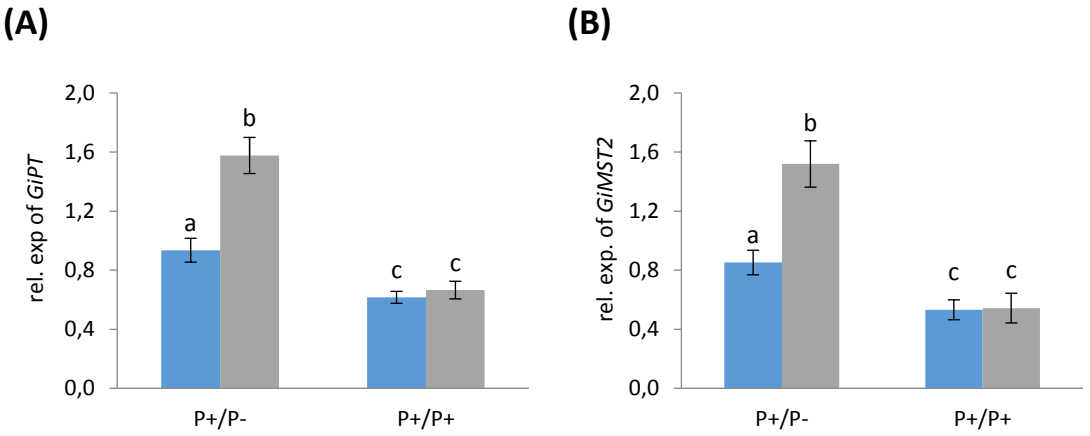

Figure III.4: Relative expression of the fungal transporters *GiPT* (A) and *GiMST2* (B) in the roots of the first split root experiment. Plants were 12 weeks old, with 6 weeks in the split root system. RNA was extracted from the root material. Relative *GiPT* transcript levels were determined using RT-qPCR analysis with *GiRNS* as a normalizer. P+/P-: plants fertilized with 0.3 mM Pi on the P+ side and no phosphate on the P- side; P+/P+: plants fertilized with 0.3 mM Pi on both sides. Each pair of columns represents a split root system with one column per compartment. Data are given as mean values +/- SE (n=6; P+/P+ n=10; P+/P-, n=25). Different letters indicates significant differences ($p<0.05$).

3.2 Influence of Differences in Phosphate Accessibility in the Split Root Experiment

The second split root experiment focused on the effect of a difference in availability of phosphate sources. A phosphate solution as in the first experiment was chosen as an easily accessible source, and apatite served as less accessible phosphate source. I wanted to determine if the accessibility of the phosphate has an influence on the expression pattern of both phosphate and sugar transporter genes in tomato and *R. irregularis*.

3.2.1 Phenotypical and Physiological Changes in Response to Apatite Fertilization

After three weeks, there were huge phenotypic differences between the plants receiving phosphate in the nutrient solution and the plants with only apatite as a phosphate source. The AA and AN plants were smaller and appeared less healthy. The differences between phosphate solution and apatite fed plants became more pronounced in the next three weeks (Fig. III.5). The AA and AN plants had a significantly smaller root and shoot weight than the PA, PP and PN plants nourished by phosphate solution (Table III.1). Furthermore, the shoot weight of the PP plants was significantly higher than for the PN plants and their root weight was significantly higher than the root weight of the N side of the PN plants. Differences in root fresh weight between the two sides of each treatment were not significant (Table III.1).

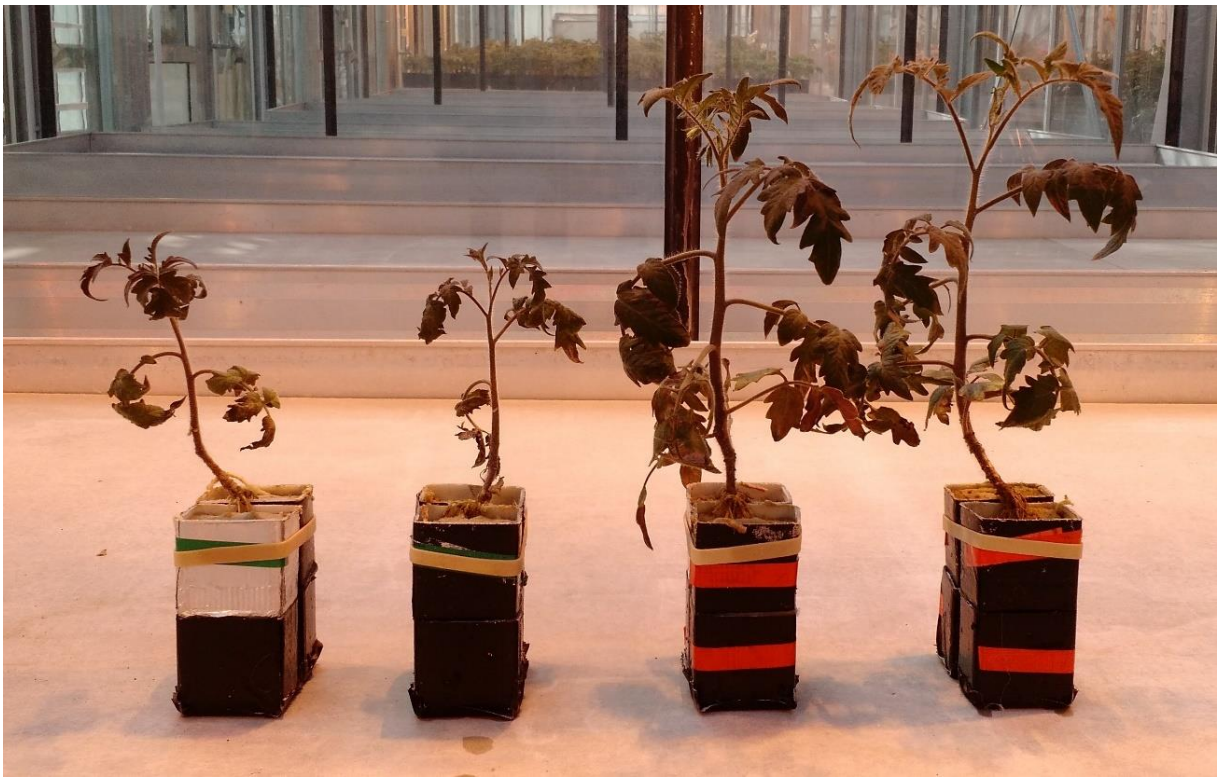

Figure III.5: Tomato plants from the second split root experiment after 4 weeks in the experimental set-up. Left side with green color: AN plants; right side with red color: PP plants

The root phosphate status shows that the phosphate solution might offer an advantage over apatite as a P source for the tomato plants. Plants receiving only apatite showed the lowest phosphate values, though the differences were only significant compared to the P side of the PA and PN plants. The same can be seen in the shoots with the highest phosphate content in the PP plants and a significantly lower phosphate content in the AA and AN plants (Table III.1).

The percent of carbon in the leaves was also significantly higher in the plants fertilized with phosphate solution compared to the AA and AN plants, whereas the percent of nitrogen was lower in these plants, though not significant (Table III.1).

Tabel III.1: Weights, phosphate, nitrogen and carbon content of shoots and roots from the second split root experiment. PP: plants receiving phosphate on both sides; PA: plants receiving phosphate on one and apatite on the other side; PN: plants receiving phosphate on one and no phosphate on the other side; AA: plants receiving apatite on both sides; AN: plants receiving apatite on one and no phosphate on the other side. Data are given as mean values +/- SD (n=6). Different letters indicates significant differences (ANOVA, post-hoc Test: Tukey; $p<0.05$).

Sample	PP	PA	PN	AA	AN
Shoot dry weight in g	2.84^{a} ± 0.246	2.34^{ab} ± 0.459	2.18^{b} ± 0.505	0.81^{c} ± 0.183	0.61^{c} ± 0.230
Root fresh weight in g	a: 3.09^{a} ± 0.230	A: 2.57^{ab} ± 0.550	N: 1.84^{b} ± 0.421	a: 1.07^{c} ± 0.595	A: 0.85^{c} ± 0.545
	b: 3.17^{a} ± 0.565	P: 2.61^{ab} ± 0.933	P: 2.79^{ab} ± 0.812	b: 0.95^{c} ± 0.665	N: 0.71^{c} ± 0.324
Shoot phosphate content in mg/g	0.85^{a} ± 0.062	0.71^{b} ± 0.065	0.68^{b} ± 0.062	$0,31^{c}$ ± 0,039	0.40^{c} ± 0.081
Root phosphate content in µg/g	a: 7.35^{ab} ± 1.320	A: 5.42^{ab} ± 3.148	N: 6.63^{ab} ± 2.124	a: 5.04^{b} ± 1.194	A: 4.17^{ab} ± 6.896
	b: 8.57^{ab} ± 1.147	P: 10.19^{a} ± 3.509	P: 9.94^{a} ± 4.395	b: 3.65^{b} ± 0.589	N: 5.03^{ab} ± 0.862
Shoot nitrogen content in %	2.29^{a} ± 0.208	2.61^{a} ± 0.310	2.74^{a} ± 0.358	3.59^{a} ± 0.217	3.94^{a} ± 0.303
Shoot carbon content in %	39.26^{a} ± 0.487	38.49^{a} ± 0.489	38.06^{a} ± 0.549	34.57^{b} ± 1.351	34.68^{b} ± 1.536

3.2.2 Phosphate Transporter Gene Expression in Response to Apatite Fertilization

As in the first experiment, the qPCR data showed a similar expression pattern for *LePT4* and the fungal marker genes. There was a negative effect of apatite on the mycorrhization as well as the *LePT4* expression (Fig III.6A). For the fungal marker gene as well as for *LePT4*, the expression in the plants fertilized only with apatite was significantly lower than in the plants fertilized with phosphate (Fig. III.6). The differences between the sides of the treatments were not significant.

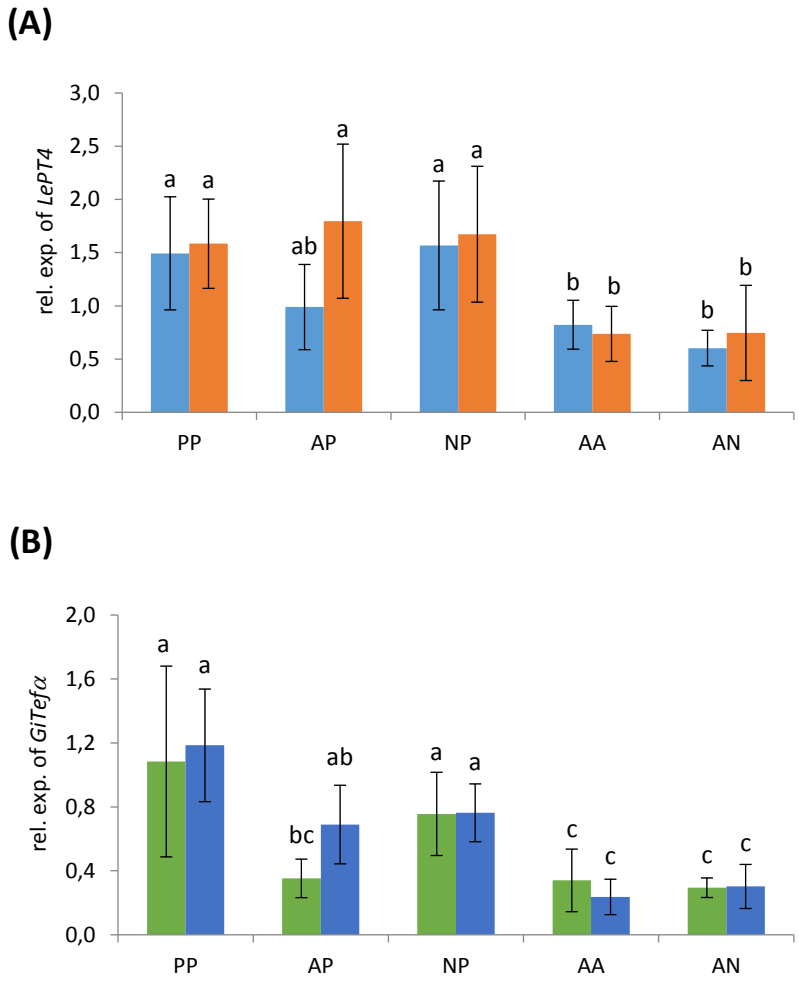

Figure III.6: Relative expression of the phosphate transporter *LePT4* (A) and the fungal marker gene *GiTefα* (B) in the roots of the second split root experiment. Plants were harvested after 12 weeks, with 6 weeks in the experimental design. Relative *LePT4* and *GiTefα* transcript levels were determined using RT-qPCR analysis. *LeTefα* was used as a normalizer. PP: plants receiving phosphate on both sides; PA: plants receiving phosphate (P) on one and apatite (A) on the other side; PN: plants receiving phosphate (P) on one and no phosphate (N) on the other side; AA: plants receiving apatite on both sides; AN: plants receiving apatite (A) on one and no phosphate (N) on the other side. Each pair of columns represents a split root system with one column per compartment. Left bars: side with the first letter of the combination; right bars: side with the second letter of the combination below. Data are given as mean values +/- SD (n=5). Different letters indicate significant differences between the mean value of two treatments (A and C) or two sides (B) ($p<0.05$).

3.2.3 Sugar Transporter Expression Gene in Response to Apatite Fertilization

The lower gene expression in the apatite fertilized plants compared to the phosphate plants can also be seen for *SlSUT1*, but the differences were only significant for the AA plants compared to the PP plants and the P side of the AP plants and for the A side of the AN plants compared to the PP plants (Fig III.7A). No significant differences could be found for the expression of *SlSUT2* (Fig III.7B).

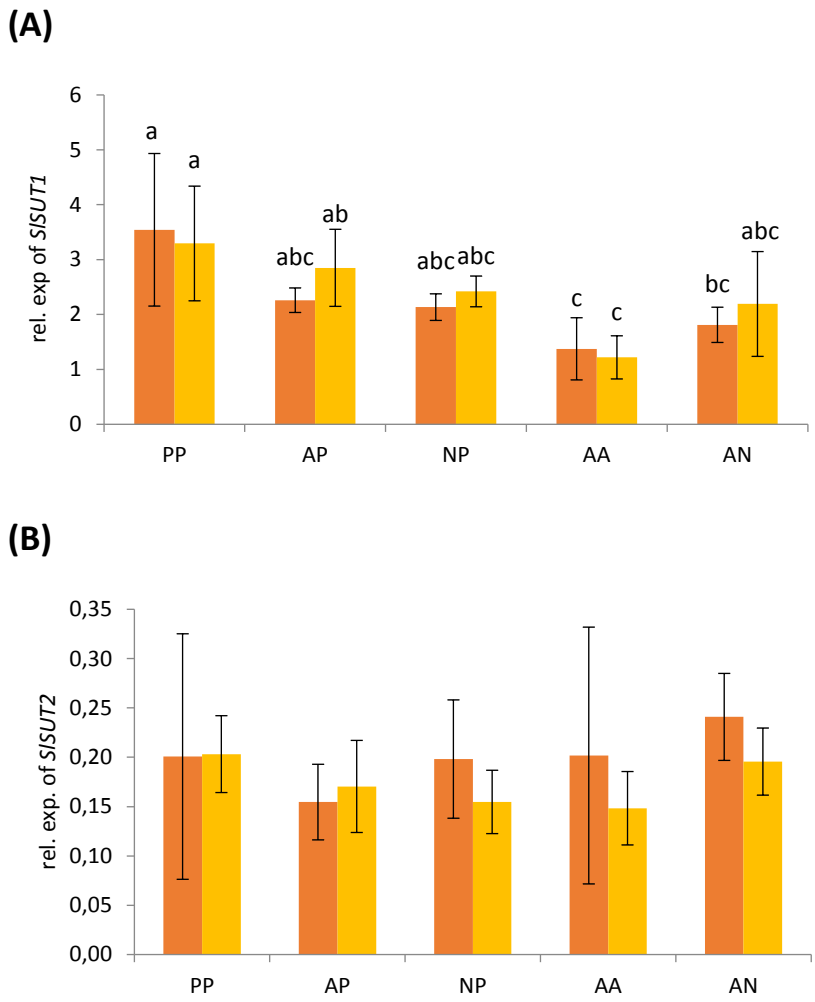

Figure III.7: Relative expression of the plant sucrose transporters *SlSUT1* (A) and *SlSUT2* (B) in the roots of the second split root experiment. Plants were harvested after 12 weeks, with 6 weeks in the experimental design. Relative *SlSUT1* and *SlSUT2* transcript levels were determined using RT-qPCR analysis. *LeTef*α was used as a normalizer. PP: plants receiving phosphate on both sides; PA: plants receiving phosphate (P) on one and apatite (A) on the other side; PN: plants receiving phosphate (P) on one and no phosphate (N) on the other side; AA: plants receiving apatite on both sides; AN: plants receiving apatite (A) on one and no phosphate (N) on the other side. Orange bars: side with the first letter of the combination; yellow bars: side with the second letter of the combination below. Data are given as mean values +/- SD (n=5). Different letters indicate significant differences between the mean value of two treatments (A and C) or two sides (B) ($p<0.05$).

3.2.4 Fungal Transporter Gene Expression in Response to Apatite Fertilization

The expression of *GiMST2* and *GiPT* was higher in the AA and AN plants compared to the plants receiving phosphate solution (Fig III.8). *GiPT* had a significantly higher expression in the AN plants compared to the PP plants and the P side of the PA and PN plants. For *GiMST2*, the expression in the AA plants was significantly higher than in all the treatments receiving phosphate solution. The A side of the AN plants showed also a significantly higher expression than most of the phosphate fertilized plants, with the exclusion of the A side of the PA plants. The expression pattern of the fungal genes in

this experiment is therefore comparable to the first part of the split root experiment, with a higher expression when less phosphate is available.

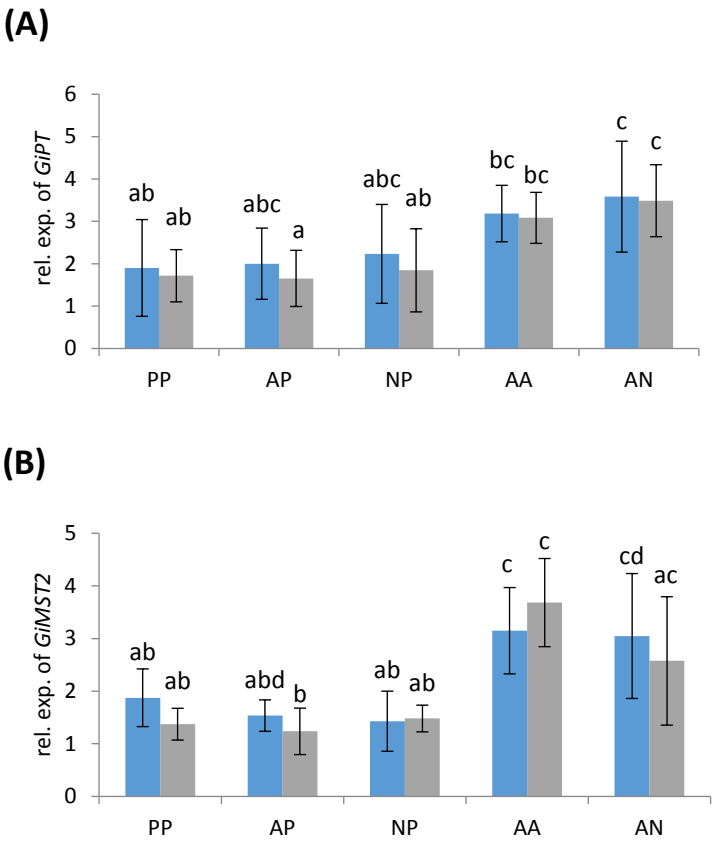

Figure III.8: Relative expression of the fungal transporters *GiPT* (A) and *GiMST2* (B) in the roots of the second split root experiment. Plants were harvested after 12 weeks, with 6 weeks in the experimental design. Relative transcript levels were determined using RT-qPCR analysis. *GiTef*α was used as a normalizer. PP: plants receiving phosphate on both sides; PA: plants receiving phosphate (P) on one and apatite (A) on the other side; PN: plants receiving phosphate (P) on one and no phosphate (N) on the other side; AA: plants receiving apatite on both sides; AN: plants receiving apatite (A) on one and no phosphate (N) on the other side. Blue bars: side with the first letter of the combination; grey bars: side with the second letter of the combination below. Data are given as mean values +/- SD (n=5). Different letters indicate significant differences between the mean value of two treatments (A and C) or two sides (B) ($p<0.05$).

4. Discussion of the Split Root Experiment with *Solanum lycopersicum*

4.1 Regulation Processes in Gene Expression in Response to Partial Phosphate Starvation

The expression of the tomato phosphate transporters *LePT4* and *LePT3* is known to be influenced by the mycorrhization of roots (Javot, Pumplin, et al. 2007; Liu et al. 2007; Balestrini et al. 2007; Gómez-Ariza et al. 2009; Nagy et al. 2009). *LePT4* is exclusively expressed in mycorrhized plants and *LePT3* shows a significant upregulation. As the expression of both transporters was significantly lower on the P- side of the split root experiment than on the P+ side, the phosphate status of the soil also seems to be a factor in the regulation of *LePT4* and *LePT3* expression. The qPCR with the fungal marker genes *GiTefα* and *GiRNS* showed the same expression pattern, and the degree of mycorrhization was confirmed by microscopy and staining techniques. Different question arise based on my data, such as how the phosphate availability influences the degree of mycorrhization and the phosphate transporter expression, and how much influence the mycorrhization has on the phosphate transporter expression in comparison to the phosphate availability.

4.1.1 Influence of the Phosphate Availability on the Degree of Mycorrhization

Several studies found a correlation between the percentage of mycorrhization and the P availability of the soil (Menge et al. 1978; Thomson et al. 1986; Schmidt et al. 2010; Bonneau et al. 2013; Balzergue et al. 2011; Amijee et al. 1989). In my experiment, the absence of phosphate leads to a decrease in mycorrhization. It has already been demonstrated repeatedly that an excessive supply of phosphate leads to a lower mycorrhization or even no mycorrhization of the plant (Menge et al. 1978; Thomson et al. 1986; Breuillin et al. 2010; Schmidt et al. 2010). This has also been shown for tomato colonized with *R. irregularis* (Nagy et al. 2009). However, the impact of the soil phosphorus status on the amount and diversity of the mycorrhizal colonization can vary between different plant and fungal species (Gosling et al. 2013). The authors found a significant reduction in the percentage mycorrhizal colonization of maize roots for high P concentrations in soil, whereas soybean and *Viola* showed no significant effect to changes in P concentrations. All species showed a change in the composition of AM communities with varying P concentrations, although the degree varied (Gosling et al. 2013). It has also been shown that even within a fungal species, the symbiotic efficiency as well as the colonization and P translocation can vary (Avio et al. 2006; Mensah et al. 2015; Munkvold et al. 2004; Lerat, Lapointe, Piché, et al. 2003; Koch et al. 2006). My experiment shows that the mycorrhization of the roots also suffers from the absolute absence of phosphate, at least after a starvation period of several weeks. The results are in accordance with a study with sunflower

infected with *Glomus etunicatum* (Koide & Li 1990). Pure sand as a substrate, with no additional phosphate, led to a very low infection rate of the plants, which increased with better phosphate availability. Koide & Li (1990) further suggested that the P status of the roots has a higher influence on the degree of mycorrhization than the shoot P status. The data from my experiment can confirm both assumptions. No phosphate in the medium led to a significant decrease in mycorrhization, but only the side of the split root under phosphate starvation was affected, independent of the shoot phosphate status. This stands in contrast to results from studies working with high P concentrations. The repressing effect of high phosphate fertilization on the mycorrhizal colonization of the roots is described to be systemic and likely influenced by the shoot phosphate status (Menge et al. 1978; Breuillin et al. 2010). Though the effect of a decrease in mycorrhization is the same for either high phosphate or the lack of phosphate, the mechanism behind this effect seems to be different. For high phosphate, a regulation through the shoot with a mobile signal, which works systemically in the roots and alters their ability to engage in mycorrhizal symbiosis, seems likely (Breuillin et al. 2010). However, for the lack of phosphate, my data suggest a different mechanism. It seems likely that the absence of phosphate has a direct impact on the growth of the mycelium. As well as the plant, the fungus needs phosphate for its own infrastructure. If nearly no phosphate is available, the growth of the fungus will be deprived. This affects the first inoculation of plants (Koide & Li 1990) as well as plants that were already mycorrhized like in my experiment. Furthermore, it seems likely that the fungus will use the little phosphate available in the substrate for its own growth instead of trading it to the plant. This could also influence the degree of colonization in the roots.

4.1.2 Influence of the Phosphate Availability on Phosphate Transporter Gene Expression

The expression pattern of *LePT4* and *LePT3* both showed a clear correlation to the soil P status, with a down-regulation on the P- side of the split roots. Other studies also found a correlation between these two factors, but in contrast generally a down-regulation of the mycorrhizal phosphate transporters in response to high P abundancy has been observed. For example, in *Petunia hybrida* inoculated with *R. irregularis*, the mycorrhiza induced phosphate transporter genes *PhPT4* and *PhPT5* were repressed in response to a high P fertilization within two days after the fertilization, whereas the described reduction of mycorrhizal colonization could only be observed after two weeks (Breuillin et al. 2010). The study suggests that the repression of the *PT* genes may be the cause of the decrease in mycorrhizal colonization (Breuillin et al. 2010). In tomato, the *LePT4* and *LePT3* expressions were also strongly down-regulated at high plant phosphorus status, despite visible colonization by AM fungi (Nagy et al. 2009). Concurrently, the contribution of the mycorrhizal P uptake pathway to the plant P status was significantly reduced (Nagy et al. 2009). A different

example is given by an in vitro study on *M. truncatula* roots colonized with *R. irregularis*, where the phosphate transporter *MtPT4* was slightly up-regulated when fertilized with a medium phosphate solution (320 µM) compared to a low phosphate solution (32 µM) (Fiorilli et al. 2013). Their study shows that even minor changes in phosphate availability can lead to changes in phosphate transporter expression. However, to this point the expression rate of these transporters when no phosphate is available has not been examined. My data suggests that the non-availability of phosphate also has a significant effect on both transporter expression patterns. Just as for the colonization, the repressing effect is not systemic, but restricts itself to the area affected by the phosphate starvation. This is again different from the regulation under high phosphate availability, where a systemic repression could be observed (Breuillin et al. 2010). The difference in regulation for both situations seems reasonable. For a high phosphate status in the shoot, a systemic down-regulation of the mycorrhizal phosphate transporters as well as the colonization is beneficial for the plant, as the fungus is no longer an advantage. In case of restricted phosphate starvation, a systemic down-regulation would lead to a disadvantage for the plant, since it is still receiving phosphate from the fungus in some root areas. My experiments are also in accordance with the observations made by Fiorilli et al. (2013). In both cases, a medium concentration of phosphate (320 µM) led to an up-regulation of the phosphate transporter compared to an even lower amount of P. This shows that the regulation of the phosphate transporter gene expression is likely to be influenced by several different signals, depending on the specific situation in the plant and its surroundings. The different regulation patterns indicate a complicated and very precise control mechanism in the symbiosis.

4.1.3 Correlation of Mycorrhization and Phosphate Transporter Gene Expression

The *LePT4* and *LePT3* expression as well as the degree of mycorrhization correlates with soil P status in my experiment, both with a decrease on the P- side of the split roots. However, the expression of *LePT4* is 3.2 fold higher for the P+ side than for the P- side, whereas the mycorrhization is only 1.5 to 2.3 fold higher on the P+ side, depending on the technique and marker gene used. Hence, the difference in the *LePT4* expression is far more distinct than the difference in the degree of mycorrhization between the two sides. I suggest that mycorrhization alone cannot fully explain the disparity in the *LePT4* expression rate and that the phosphate concentration has a direct influence on the expression. Since most mycorrhizal phosphate transporters are exclusively expressed in mycorrhized cells (Rausch et al. 2001; Paszkowski et al. 2002; Harrison et al. 2002; Nagy et al. 2005; Liu et al. 2007; Javot, Penmetsa, et al. 2007; Nagy et al. 2009; Gómez-Ariza et al. 2009), more mycorrhized cells in the root tissue should directly lead to more *LePT4* transcripts. The use of phosphate transporters as a marker for a functional symbiosis has also been suggested (Javot,

Pumplin, et al. 2007). However, a study in tomato found no influence of a *LePT4* knock-out mutant on the mycorrhization rate, but a decrease of the beneficial effect of mycorrhiza compared to wild type plants (Xu et al. 2007). Furthermore, Nagy et al. (2009) examined a situation where no *LePT4* transcripts could be detected, although a colonization by AM fungi could be observed. This strengthened their theory that the repression of the mycorrhizal transporter genes are causing the inactivation of the mycorrhizal P uptake pathway under high P fertlilization in favor for the direct P uptake pathway. In their study with *Petunia hybrida* colonized with *R. irregularis*, Breuillin et al. (2010) found a repression of both colonization and PT genes, but also not in proportion. The repression of *PhPT4* and *PhPT5* expression in response to a high shoot phosphate status was stronger than the decrease in colonization, hence the authors suggested a direct effect of the phosphate status on the transporter expression (Breuillin et al. 2010). These examples show that mycorrhization and *LePT4* expression can differ from each other, despite the often observed correlation mentioned above. This underlines my findings and sustains the hypothesis that the enhanced *LePT4* expression in my experiment is due to the variation in soil P status instead of solely originating from the difference in mycorrhization.

4.1.4 Regulation of the Sugar Transporter gene Expression in Response to Partial Phosphate Starvation

Sugar transporters in plants are key components in the long distance transport of sugars from source to sink organs, and it has been shown that the roots of mycorrhizal plants act as an important sink system for photosynthetic products (Wright, Read, et al. 1998; Doidy et al. 2012). Therefore, it can be assumed that they also play a role in the regulation processes of the symbiosis. I wanted to determine if the expression patterns of the mycorrhiza-induced sugar transporters *SlSUT1* and *SlSUT2* change according to the phosphate availability in the soil and thereby to the possible Pi transfer from the fungus to the plant.

I hypothesized a downregulation of both transporters if no phosphate is available, compared to a low amount of phosphate in the soil. Unexpectedly, the expression of *SlSUT1* as well as *SlSUT2* was not influenced by the phosphate status of the soil. No significant differences between the P+ side and the P- side of the roots could be observed. Hence, it is also unlikely that the expression of the sugar transporters is directly influencing the mycorrhizal phosphate transporter expression or *vice versa*. My data is in accordance with a study from Ge et al. (2008), in which the phosphate status of the plant had no influence on the expression of *SlSUT1* and *SlSUT2* in non-inoculated plants. Their study also showed a difference in *SlSUT1* expression between inoculated and non-inoculated plants under

high P fertilization, suggesting that its expression is influenced by mycorrhizal signals, which are not directly dependent on the plant phosphate status.

4.1.5 Influence of Mycorrhization on *SlSUT1* and *SlSUT2* Expression

The expression of both transporters in the nonmycorrhized plants was also different from my expectations. Although it is stated in the literature that both sugar transporters, especially *SlSUT1*, show an upregulation in the leaves or roots due to mycorrhization (Boldt et al. 2011), my findings could not confirm this. *SlSUT2* showed no difference in expression between the mycorrhized and nonmycorrhized plants, whereas *SlSUT1* was even significantly downregulated in the mycorrhized plants. However, both observations have been made before. Tomato plants inoculated either with *R. irregularis* or *G. caledonium* showed a decrease in *SlSUT1* expression compared to nonmycorrhized plants (Ge et al. 2008). The same study also found no effect of a mycorrhization on the expression of *SlSUT2*. My data supports those results. Different explanations are possible for the deviation of the results from Boldt et al. (2011). Although my data suggests that the availability of phosphate did not influence *SlSUT1* expression, as described above, there is still a possibility that the phosphate status of the root had an influence on *SlSUT1*. The nonmycorrhized plant roots in my experiment had significantly less phosphate than the mycorrhized roots, and the other trends in phosphate concentration in the roots were also mirrored in the *SlSUT1* expression (compare Fig. III.1C and III.3A). The plants in the study from Boldt et al. (2011) received with 0.13 mM P slightly more than my plants. It has been stated that Pi starvation leads to an increase in carbon allocation to the roots (Liu et al. 2005; Hermans et al. 2006; Hammond & White 2008; Lemoine et al. 2013). The One Arm Experiment (section 5.1) supports this theory, as the plants receiving either no phosphate or apatite had a significantly higher cumulative C gain in the outer compartment then the phosphate treated plants. This reallocation could be accompanied by an increase in sugar transporter expression. Furthermore, it has been shown that an overexpression of the analogue transporter *SoSUT1* in potato led to an increase in mycorrhization under high phosphate, but not under low phosphate conditions (Gabriel-Neumann et al. 2011). This suggests a link between *SUT1* expression and phosphate status at least in mycorrhized plants. Therefore, the possibility of an up-regulation due to the severe phosphate starvation in the roots seems still plausible. Another explanation could be that the degree of mycorrhization, or more likely the species of the fungal symbiont, is of importance for the *SlSUT1* expression, as my plants showed a relatively low mycorrhization with 15% of arbuscules, whereas Boldt et al. (2011) described the colonization in their experiment as intense. Furthermore, Boldt et al. (2011) used the fungus *Glomus mossae* while my experiments were carried out with *R. irregularis*. Ge et al (2008) inoculated their plants with *R. irregularis* and *G. caledonium*. Therefore,

the expression of the gene could vary between these species. It has been shown that a different expression pattern of mycorrhiza related genes for different fungal species is possible (Feddermann et al. 2008; Feddermann et al. 2010). For example, Ge et al. (2008) showed that the putative monosaccharide transporter *LeST3* was upregulated in roots and leaves of plants inoculated with *R. irregularis*, whereas plants inoculated with *G. caledonium* showed a decreased expression. The aforementioned factors could be responsible for the different and unexpected expression pattern of *SlSUT1* and *SlSUT2* in my study, with a species-related expression as the most likely theory. Nevertheless, additional experiments would be useful to fully understand the role and regulation of these two sugar transporters.

4.1.6 Regulation of the Fungal Transporter *GiPT* in Response to Partial Phosphate Starvation

Although the research is not yet as extensive as in the plant symbiont, there are also many transporters involved on the fungal side of the symbiosis, including phosphate and sugar transporters. In this study, I wanted to further determine the expression pattern of the fungal transporter genes *GiPT*, a phosphate transporter mostly active in the extraradical mycelium (Maldonado-Mendoza et al. 2001), and *GiMST2*, a hexose transporter expressed in the intraradical mycelium (Helber et al. 2011). Against my hypothesis, the expression of the both fungal transporter genes *GiPT* and *GiMST2* was higher on the P- side of the split root system compared to the P+ side. The hypothesis was based on findings from other experiments, where the expression of *GiPT* in the extraradical mycelium was influenced by the phosphate level of the surrounding medium as well as of the plant roots (Maldonado-Mendoza et al. 2001; Olsson et al. 2006; Fiorilli et al. 2013). However, my results derive from the intraradical mycelium. Hence, a different expression pattern than in studies conducted with the extraradical mycelium is not exceptional. The intraradical mycelium was not in the focus of studies with fungal phosphate transporters. A study with *GmosPT* from *Glomus mossae* showed that the expression level of this gene changes in the extraradical mycelium due to the amount of phosphate present in their surroundings, whereas the expression in the intraradical mycelium showed a constant expression level independent from external phosphate concentrations (Benedetto et al. 2005). The presence of *GmosPT* transcripts in arbusculated plant cells was confirmed with laser microdiessection techniques by Balestrini et al. (2007) and Gómez-Ariza et al. (2009). Harrison & van Buuren (1995) could also detect transcripts of the fungal phosphate transporter *GvPT* in the intraradical mycelium in *M. truncatula* roots colonized by *G. versiforme*, although in extremely low amounts. This suggests that the presence of phosphate transporter transcripts in the intraradical mycelium differs between species. Accordingly, my results give new insight in the expression pattern of *GiPT* from *R. irregularis*. The presence of *GiPT*

transcripts in arbusculated cells of *M. truncatula* has already been shown (Tisserant et al. 2012; Fiorilli et al. 2013). A possible explanation for the upregulation in response to the absence of P in the soil could be a Pi-starvation induced expression of *GiPT* in the intraradical mycelium. Moreover, it has been suggested that a competition for phosphate occurs at the plant-fungal interface. The abundance of phosphate transporter transcripts in the intraradical mycelium might indicate a control of the fungus over the amount of phosphate delivered to the plant through an uptake of Pi by the fungus concurrent to its efflux (Benedetto et al. 2005; Balestrini et al. 2007; Fiorilli et al. 2013). Fiorilli et al. (2013) also examined *GiPT* expression in the intraradical mycelium under different P conditions. A slightly higher P concentration (320 µM compared to 32 µM) led to a downregulation of *GiPT* expression inside the plant cells. My findings are in accordance with this experiment, since I could also observe a down-regulation in response to the higher P concentration. The higher expression of *GiPT* under Pi starvation in my study would further support the theory of a competition for P at the periarbuscular membrane. An upregulation due to Pi-starvation would also be in line with this explanation. A similar mechanism in arbusculated cells has also been proposed for the mycorrhizal trading with nitrogen (Pérez-Tienda et al. 2011).

4.1.7 Correlation of *GiMST2* and *LePT4* Expression under Phosphate Starvation

Based on the literature, my hypothesis was that *GiMST2*, as well as *LePT4*, would be down-regulated if no phosphate was available to the fungus in the soil. The expression of *GiMST2* is described to be correlated to the expression of the *PT4* gene in potato as well as in *M. truncatula* (Helber et al. 2011). This was shown in a time course experiment after the initial inoculation with the fungus as well as in a second experiment under the influence of different phosphate fertilizations. A fertilization with a high amount of phosphate led to a decrease in *PT4* as well as in *GiMST2* expression (Helber et al. 2011). In my experiment, *GiMST2* was upregulated in response to Pi starvation, whereas *LePT4* was downregulated. A positive correlation between the expression of the two genes could not be observed. This is in line with results from Fiorilli et al. (2013), where *GiMST2* expression was downregulated in response to medium phosphate conditions (320 µM) compared to low phosphate conditions (32 µM), whereas *MtPT4* showed an upregulation for the higher phosphate concentration. The regulation of *GiMST2* in response to the total absence of phosphate was not tested in both other studies, and it is certainly possible that *GiMST2* expression correlates with *PT4* expression under high phosphate conditions, but not under Pi starvation or low to medium concentrations. This would indicate that different signals are involved in the regulation of *GiMST2* and *PT4* expression, although a link between the symbiotic phosphate delivery and the *MST2* expression, as suggested by Helber et al. (2011), is still possible. It was also theorised that other fungal sugar transporters are involved in

the C for P exchange, hence *GiMST2* expression might not be the optimal marker for the plant-fungal C flux (Fiorilli et al. 2013).

4.2 Apatite as a Phosphate Source in the Split Root Experiment

In the second split root experiment, I wanted to test the influence of the accessibility of the phosphate source. A phosphate solution was used again as an easy accessible source. As a source with lower accessibility, apatite was chosen. Apatite is the primary mineral phosphate source in natural soils (Smeck 1985). Plants cannot access the phosphate directly, but it is released through weathering processes. The weathering of apatite is enhanced by soil acidity (Smeck 1985), especially by oxalic acid (Wallander 2000). It is believed that ectomycorrhizal fungi (EM) stimulate the uptake of phosphate from apatite, either by a pH reduction of the soil through exudation of organic acids (Cromack et al. 1979; Entry et al. 1994; Graustein et al. 1977; Griffiths et al. 1994; Wallander et al. 1997; Fox & Kamprath 1970) or by a generally greater ability to absorb dissolved phosphate (Wallander et al. 1997; Wallander 2000). If the same is possible for AM fungi is still unknown, but the question arose (Koele et al. 2014).

4.2.1 Phenotypical and Nutritional Responses to Apatite Fertilization in *Solanum lycopersicum*

After three weeks in the experiment, obvious differences in the plants fertilized with the phosphate solution compared to the plants with apatite in the substrate could be observed. The differences grew more distinct during the next three weeks. At the harvesting point after six weeks, the plants receiving only apatite as a phosphate source were half the size of the phosphate fertlilized plants and appeared overall unhealthy. The fresh and dry weight of shoots and roots were also significantly lower, as well as the phosphate status of leaves and roots. In the roots, there were also distinct differences between the P+ side of the split root system and the P- or apatite side. Regarding the literature, these findings are not very surprising. Apatite is a rock mineral, and phosphate is released through weathering and acidification of the soil. However, weathering is a very slow process, which takes place over decades. The experiment shows that in six weeks and under the controlled conditions in a greenhouse, the phosphate cannot be released in a sufficient amount and is therefore not accessible for the plant. Other experiments with apatite as the solitary phosphate source were conducted over a period of 30 or 31 weeks (Wallander et al. 1997; Wallander 2000). Although there is the possibility of active weathering through the fungi (Koele et al. 2014), the AM fungus in my experiment is either not able to mine the apatite directly or indirectly by exuding low-molecular

organic acids or it is a very slow process, which cannot contribute sufficiently to a healthy phosphate status of the plant in this limited amount of time. In future experiments, it might be helpful to include control plants which receive no phosphate on both sides. This option was discussed previous to the experiment, but dismissed because of the unlikeliness that the plants would have survived the time of the experiment without phosphate. Furthermore, a sufficient mycorrhization seemed also not possible without a phosphate source on both sides. In retrospective, it seems likely that the plants would have survived, albeit in an unhealthy condition. Without these controls, it is now difficult to decide if the plant or the fungus could gain phosphate from the apatite at all. Overall, the phosphate content of the shoots receiving apatite on both sides is even slightly lower than that of the plants with apatite on just one side. This would suggest that there was no contribution to the plant phosphate from the apatite. For the fresh and dry weight of roots and shoots, there is a small tendency towards a higher weight for the AA plants, but this can be incidental. The root phosphate content also gives no further insight in this question. Furthermore, the phosphate-fertilized plants have a significantly higher carbon content than the apatite-fertilized plants. It might be possible that the lower carbon content can be ascribed to a lower capability of photosynthesis of these plants due to smaller and less leaves. The leaves also had a darker, slightly violet color because of the phosphate deficiency, which can also decrease the photosynthesis rate. Another explanation could be a carbon allocation in the apatite-fertilized plants from the shoots to the roots, since this has been described in plants under Pi starvation (Liu et al. 2005; Hermans et al. 2006; Hammond & White 2008; Lemoine et al. 2013).

4.2.2 Correlation of *LePT4* Expression with Mycorrhizal Status and Phosphate Accessibility

To determine the possible changes in the expression pattern of the transporter genes involved in the symbiosis, I also extracted RNA from the plant roots. As in the first experiment, the expression of *LePT4* is lower in plants receiving less phosphate, here the apatite-fertilized plants. Surprisingly, the expression of *LePT4* for the PN plants is nearly the same on both sides. The same can be seen for the expression of the fungal marker. However, for the PA plants, there is a significantly higher expression on the P+ side compared to the apatite side for both genes. Overall, the expression levels of both genes show a very similar pattern. This shows again the general correlation between the expression of *LePT4* and the mycorrhizal status of the plant. Nonetheless, the differences between the treatments are far more distinct for the fungal marker than for *LePT4*. The expression of *GiTefa* is nearly 4 fold higher for the PP plants than for the AA or AN plants, whereas the *LePT4* expression is just 2 fold higher for the same treatments. This indicates that the *LePT4* expression is not only influenced by the mycorrhization, as has already been shown in the first experiment. Still, in this part

of the experiment, it is quite complex to distinguish between the effect of the mycorrhization and the effect of the phosphate starvation on the *LePT4* expression. The different treatments in the second experiment made it necessary to reduce the number of replicates. Unfortunately, a clear interpretation of the data is now difficult due to high variances between the replicates and the resulting high standard deviation. In the first experiment, the number of replicates was high enough to overcome the problem of high variances between the plants, which made the data easier to interpret. Nonetheless, the general tendency towards a lower expression in plants receiving less phosphate, here the apatite-fertilized plants, is still clear.

4.2.3 Influence of Mycorrhization and Phosphate Accessibility on *SlSUT1* Expression

The sugar transporter *SlSUT1* shows a tendency towards a higher expression in the plants fertilized with phosphate solution compared to the apatite plants, which can also be seen for the mycorrhizal marker *GiTefa* and the phosphate transporter *LePT4*. This could be interpreted as a correlation between *SlSUT1* expression and mycorrhizal status of the roots. In part one of the experiment, I found an upregulation of *SlSUT1* in nonmycorrhized plants compared to mycorrhized ones, which has been observed before (Ge et al. 2008), although the opposite regulation has also been described (Boldt et al. 2011). Different possible reasons for the contrary regulation have been discussed previously. In the second part of the experiment, nonmycorrhized plants were not included in the controls due to technical reasons. Therefore, this experiment cannot contribute to the earlier discussion. However, none of the two studies mentioned above compared the *SlSUT1* expression in plants with a different degree of colonization. My results suggest either a correlation with the degree in mycorrhization or with the phosphate status in the plant or the surrounding soil. As the absence or presence of mycorrhization has an effect on *SlSUT1* expression, though not always the same one, it seems possible that different degrees of mycorrhization can lead to changed *SlSUT1* expression as well. A lower mycorrhization and therefore a smaller fungal contribution to the phosphate status of the plant would lower the demand for sugar in the plant roots compared to better mycorrhized roots and hence could be linked to a lower expression of *SlSUT1*. Nevertheless, the difference in expression in my experiment is only significant for the PP plants compared to the AA or AN plants. The PN and PA plants just show a significant difference to the AA plants, not to the AN plants. Furthermore, the P+ and P- side of the plants in the first part of the experiments showed a significant difference in mycorrhization, but not in *SlSUT1* expression. Therefore, it is not clear that the degree of mycorrhization is the controlling factor of *SlSUT1* expression. The second possibility would be an influence of the phosphate status of the roots or the soil, which was also discussed in part one of the experiment. Although the results from the second part seem to strengthen this theory, the

differences in *SlSUT1* expression are contrary to the results from part one, where the roots with less phosphate tended to have a higher expression. The difference between the P+ and P- side in the first experiment was not significant, though, whereas the differences in the second part are more distinct. In the literature, the only comparison of *SlSUT1* expression under different phosphate availabilities was in nonmycorrhized plants, where no differences could be observed (Ge et al. 2008). Hence, my data cannot be easily compared to other results. More research in this direction would be necessary to unravel the factors involved in controlling *SlSUT1* expression. It is also possible that another factor is involved in regulating *SlSUT1* expression, which is not under control in this experiment.

4.2.4 Influence of Mycorrhiztion and Phosphate Accessibility on *SlSUT2* Expression

The expression of *SlSUT2* was also determined in both the first and the second experiment. However, there were no significant differences between any of the treatments, neither in the first nor the second experiment. Furthermore, the variances within each treatment were relatively high, which makes it difficult to find a correlation between the controlled factors. Although a role of *SlSUT2* in the mycorrhizal symbiosis was shown (Bitterlich et al. 2014), the experiments with this transporter so far show no regulation in the roots in response to mycorrhization (Ge et al. 2008; Boldt et al. 2011). It is possible that *SlSUT2* regulation happens in the leaves, where an increase in expression in mycorrhized plants was observed (Boldt et al. 2011). The regulating process could also work in the other direction. Plants with reduced *SlSUT2* expression in the roots (*SlSUT2* antisense plants) showed an increase in mycorrhization over several generations (Bitterlich et al. 2014). The same plants had no positive mycorrhizal effect compared to the wild type plants. Thus, *SlSUT2* expression could be a possibility for the plant to control the trading process in the symbiosis. SlSUT2 is located in the periarbuscular membrane, and the study suggests that it transports sucrose from the periarbuscular space back into the cytoplasm. A downregulation of *SlSUT2* would therefore lead to a greater supply of carbohydrates for the fungus and an increase in colonization (Bitterlich et al. 2014). Though my experiment cannot directly confirm this theory, the results are also not in conflict with this model. Furthermore, it is proposed that the regulation of the SlSUT2 protein level might be post-transcriptional or post-translational, since an increase in the protein level in mycorrhized roots could be observed (Bitterlich et al. 2014), without an increase in the expression level (Boldt et al. 2011). My results would strongly support this hypothesis.

4.2.5 Influence of Different Phosphate Accessibilities on *GiPT* and *GiMST2* Expression

As in the first part of the experiment, I also wanted to determine if the changes in the phosphate source had any influence on the expression of the fungal transporters *GiPT* and *GiMST2*. Both genes show a higher expression in the roots of the apatite fertilized plants, which is more pronounced for *GiMST2*. This confirms the results of the first experiment, which also showed a higher expression of *GiPT* and *GiMST2* on the P- side compared to the better fertilized P+ side of the roots. The nutritional data shows that the plants fertilized with apatite have a lower phosphate status, hence the apatite fertilized plants or root sides can be compared with the P- side of the plants in the first experiment. The data from the second experiment is in line with the findings of the first part of the experiment and supports their interpretation. *GiPT* shows a higher expression in the intraradical mycelium in response to Pi starvation, in contrast to its observed expression in the extraradical mycelium. It seems possible that the transporter is involved in a competition for phosphate at the periarbuscular membrane, as suggested in the literature (Benedetto et al. 2005). An upregulation due to Pi starvation is also plausible. For *GiMST2*, a positive correlation with the *PT4* expression could not be observed, contrarily to other findings in the literature (Helber et al. 2011). *GiMST2* shows a higher expression in the plant treatments where a decrease in *LePT4* expression could be observed. As with *GiPT*, this supports the results from the first part of the experiments as well as their interpretation.

5. Results of the One Arm Experiment

The aim of the One Arm Experiment was to determine the differences between the fungal uptake of different phosphate sources. I compared phosphate solution as an easy accessible source with apatite, a mineral phosphate source, and phytate, an organic phosphate source, both of which are not as easily available for the fungus as pure phosphate. The plants were harvested at different time points over a period of 24 weeks to investigate the changes in parameters over time. The harvesting points were 6, 9, 13, 17, 21, and 24 weeks after the transplantation of the plants. Due to the unhealthy state of the plants at the last two harvesting points and the first results, the analysis of the data was mostly done with data from the first four harvesting points. Different parameters, as the CO_2 efflux throughout the experiment, the nutritional status of the plants at the harvesting point, the RNA expression in the roots, and the PLFA and NLFA composition in roots and sand, were measured and compared. The results from the CO_2 measurement, the phosphate status of the plant and the PLFA and NLFA measurement will be presented in short. A more detailed version of the results will be presented by Alberto Andrino. This thesis will focus on the results of the gene expression measurement of the mycorrhizal phosphate and sugar transporter genes of tomato and their correlation with the controlled and measured factors in the experiment.

5.1 Carbon Investment of the Plant in Response to Different Phosphate Sources

For the control plants, which received no phosphate source in the outer chamber, there was no P gain by the mycorrhizal plant over the time of 112 days (Fig. V.1). The CO_2 respiration per mol of organic carbon was similar as for the phosphate and phytate treatment (Fig. V.2), which is probably due to the high microbial content in the outer chamber, estimated as the PLFA microbial biomass (Fig. V.3). The control showed a higher percentage of the bacterial PLFA biomarker (Fig. V.4) than the other treatments, and a lower percentage of the fungal PLFA biomarker (Fig. V.5), especially after 112 days.

The plants with phosphate as a P source gained up to 1.6 mg P from the outer chamber (Fig. V.1). The P gained after 56 days was significantly more compared to the apatite treatment, but less than in the phytate treatment. The plants respired significantly less CO_2 per mol of organic carbon than the apatite plants, and had in general the lowest CO_2 respiration of all treatments (Fig. V.2). The total microbial biomass as well as the fungal and bacterial PLFA biomarkers were comparable to the control plants (Fig. V.3-V.5). The plants kept a constant investment into the fungus, which was lower than for the plants with apatite and phytate as a phosphate source (Fig. V.6).

The plants with phytate as the phosphate source gained up to 1.3 mg P from the outer chamber, with a significantly higher value than the phosphate and apatite plants after 56 days. After 112 days, the gained P was significantly less than for the apatite plants (Fig. V.1). The CO_2 respiration (Fig. V.2) was comparable to the phosphate treatment. The total microbial biomass (Fig. V.3) was significantly lower than for the control plants at day 35, but the same as the control at day 112. At day 84, it was higher than for both phosphate and apatite. The sand in the HC showed the lowest amount of bacterial PLFA biomarker (Fig. V.4) and the highest amount of fungal biomarker for days 35, 56 and especially 84 (Fig. V.5). At day 112, the fungal biomarker was less than for apatite, but still higher than for the phosphate plants. After 56 days in the experiment, the phytate plants invested 4 to 5 times more into the fungal biomass to gain the same amount of phosphate than the phosphate treated plants (Fig. V.6).

The plants with apatite as a phosphate source gained up to 1.6 mg P from the outer chamber with a significantly lower value than the phosphate and phytate plants after 56 days and a significantly higher value than the phytate plants after 112 days (Fig. V.1). The CO_2 respiration showed the highest rate of all plant treatments with significantly higher values for days 56 and 84 (Fig. V.2). The total microbial biomass was comparable to the phosphate and phytate treatment, with a significantly higher biomass after 56 and 112 days (Fig. V.3). The bacterial PLFA marker showed nearly the same amounts than for the phosphate plants (Fig. V.4). The fungal PLFA biomarker was significantly lower than for the phytate plants until day 84, but reached the highest value for all treatments after 112 days (Fig. V.5). After 84 days, the plants invested 4.5 times more into the fungal biomass to gain the same amount of P than the phosphate treated plants (Fig. V.6). Overall, the microbiota was metabolically more active compared to the phosphate treated plants.

In summary, the fungus could supply the plant with equal amounts of phosphate, irrespective of the P source, but different carbon investments were necessary. The P was gained with different kinetics, which affected the carbon investment over time. However, each form of P could be used.

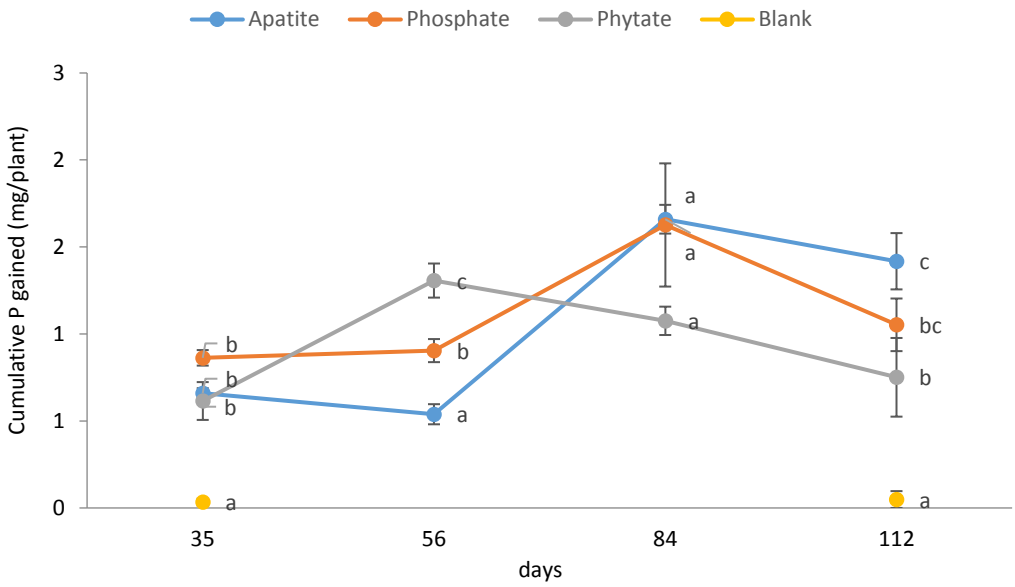

Figure V.1: Cumulative P gained from the phosphate source over the period of 112 days in the One Arm Experiment. Plants were harvested after 35, 56, 84, and 112 days in the experimental setup. Shoot and root material was dried, milled, incinerated, and taken up with HNO_3, and the phosphate status was measured with the ICP-MS. Blue: apatite treated plants; orange: phosphate treated plants; grey: phytate treated plants; yellow: control plants. Data are given as mean values +/- SE (n=3). Different letters indicate significant differences (One way ANOVA; p<0.05).

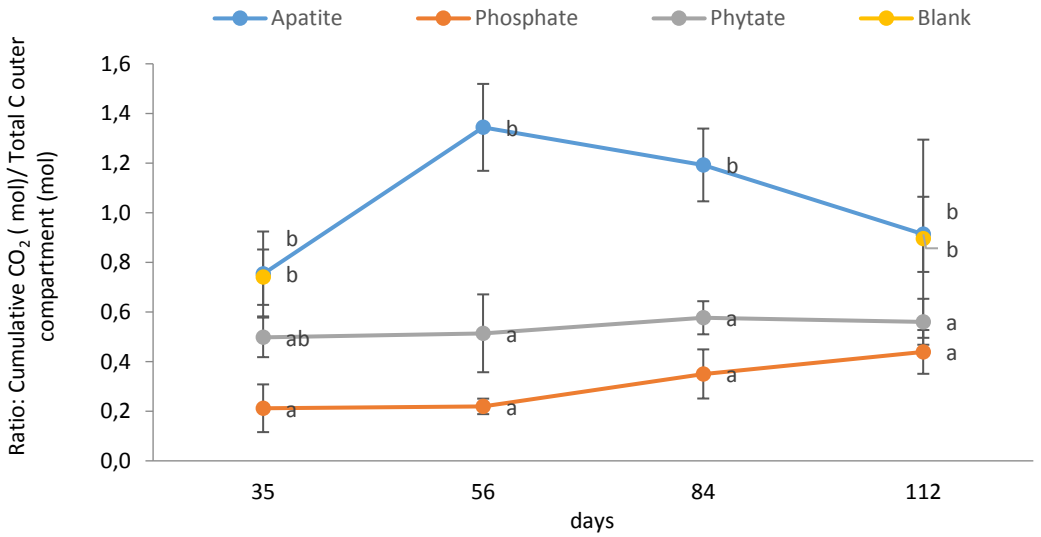

Figure V.2: CO_2 respiration over the time of 112 in the One Arm Experiment. CO_2 flux was measured weekly throughout the whole experiment with the EGM-4. Plants were harvested after 35, 56, 84, and 112 days in the experimental setup. The total amount of carbon in the outer chamber was measured with the CN analyzer after each harvest. Blue: apatite treated plants; orange: phosphate treated plants; grey: phytate treated plants; yellow: control plants. Data are given as mean values +/- SE (n=3). Different letters indicate significant differences (One way ANOVA; p<0.05).

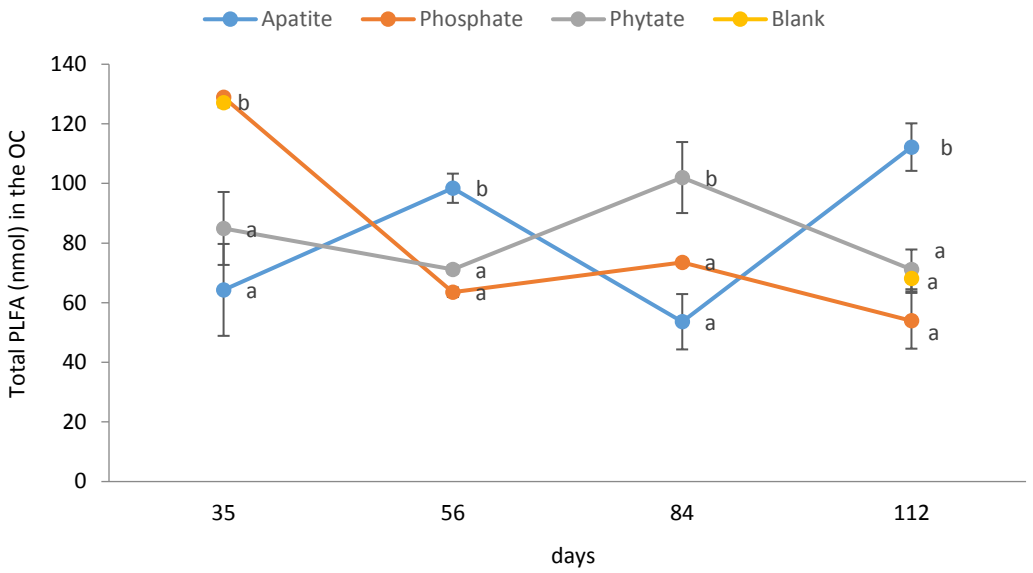

Figure V.3: Total microbial biomass in the outer chamber of the One Arm Experiment over time. Plants were harvested after 35, 56, 84, and 112 days in the experimental setup. PLFAs were extracted from the sand in the outer chamber after each harvest and measured with the elemental analyzer. Blue: apatite treated plants; orange: phosphate treated plants; grey: phytate treated plants; yellow: control plants. Data are given as mean values +/- SE (n=3). Different letters indicate significant differences (One way ANOVA; $p<0.05$).

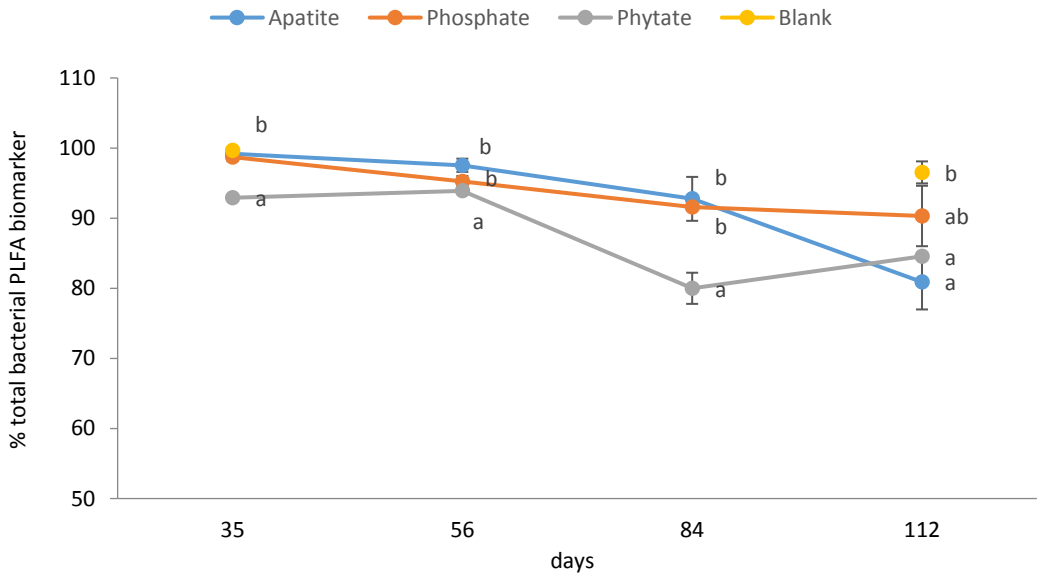

Figure V.4: Percentage of bacterial PLFA biomarker in the outer chamber of the One Arm Experiment over time. Plants were harvested after 35, 56, 84, and 112 days in the experimental setup. PLFAs were extracted from the sand in the outer chamber after each harvest and measured with the elemental analyzer. Blue: apatite treated plants; orange: phosphate treated plants; grey: phytate treated plants; yellow: control plants. Data are given as mean values +/- SE (n=3). Different letters indicate significant differences (One way ANOVA; $p<0.05$).

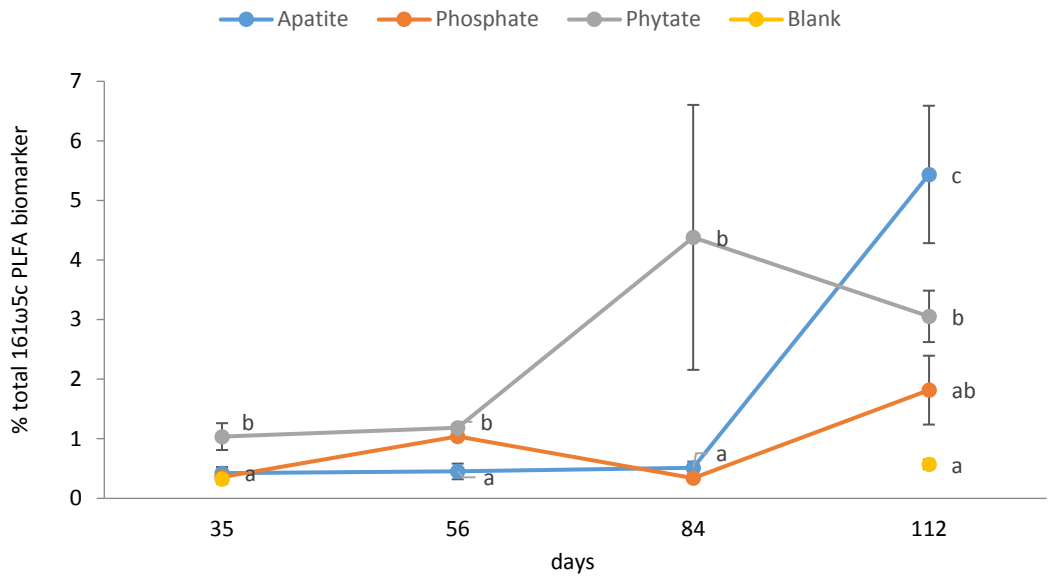

Figure V.5: Percentage of fungal PLFA biomarker in the outer chamber of the One Arm Experiment over time. Plants were harvested after 35, 56, 84, and 112 days in the experimental setup. PLFAs were extracted from the sand in the outer chamber after each harvest and measured with the elemental analyzer. Blue: apatite treated plants; orange: phosphate treated plants; grey: phytate treated plants; yellow: control plants. Data are given as mean values +/- SE (n=3). Different letters indicate significant differences (One way ANOVA; $p<0.05$).

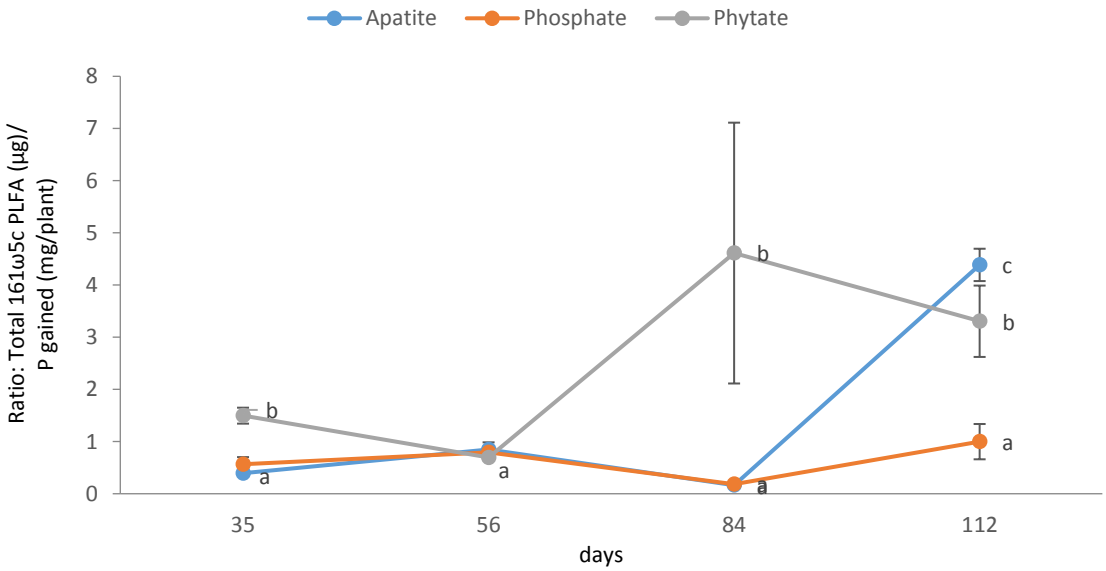

Figure V.6: Investment of the plant in the fungal biomass per mg P gained from the phosphate source depicted as the ratio between the fungal PLFA biomarker and the P gained over time in the One Arm Experiment. Plants were harvested after 35, 56, 84, and 112 days in the experimental setup. PLFAs were extracted from the sand in the outer chamber after each harvest and measured with the elemental analyzer. Shoot and root material was dried, milled, incinerated, and taken up with HNO_3, and the phosphate status was measured with the ICP-MS. Blue: apatite treated plants; orange: phosphate treated plants; grey: phytate treated plants; yellow: control plants. Data are given as mean values +/- SE (n=3). Different letters indicate significant differences (One way ANOVA; $p<0.05$).

5.2 Changes in Gene Expression in Response to Different Phosphate Sources

The results from the transporter gene expression are not as clear as for the P gain or the microbial markers. For the gene expression, all six harvesting points were measured, and the plants treated with biotite in the outer chamber instead of a phosphate source were also integrated in the analysis, as well as the control for biotite.

The expression of all transporter genes was lowest in the plants treated with biotite instead of a phosphate source. Unfortunately, there is no data available for the microbial biomass and PLFA markers of these plants. The data for the cumulative P gain of these plants are available, but in this treatment, the P was available to the plant roots as well as the hyphae, which explains a higher P gain.

5.2.1 Fungal Marker Gene Expression in Response to Different Phosphate Sources

The fungal marker gene *GiRNS* was measured to show the mycorrhizal state of the plant roots. After 35 days, the expression of *GiRNS* was relatively low in all treatments. The expression increased for day 56 and 84, with a peak for phosphate and phytate at 56 days. For day 112, the *GiRNS* expression decreased again for all treatments (Fig. V.7). The additional data for 142 and 165 days show that the *GiRNS* expression stays low for all treatments, with an exceptionally high value for phytate after 142 days. The plants treated with biotite had the lowest *GiRNS* expression in the experiment, especially at the last two harvesting points. At the last harvesting point, *GiRNS* expression was significantly different in the biotite plants compared to the apatite plants. For the other harvesting points, no significant difference between the treatments could be detected. Within each treatment, there are significant differences between the harvesting points. For apatite, there is a significant difference between 35 days and 56 days, with a lower expression after 35 days. For the phytate treatment, day 35 and 165 are significantly lower than day 56, 84 and 142. Day 142 is also significantly higher than day 112. The phosphate treated plants show a significant difference between day 56 and day 165, with a higher expression after 56 days. The expression in the biotite plants was significantly lower for days 142 and 165 compared to days 56 and 84. The controls show no significant differences between the harvesting points.

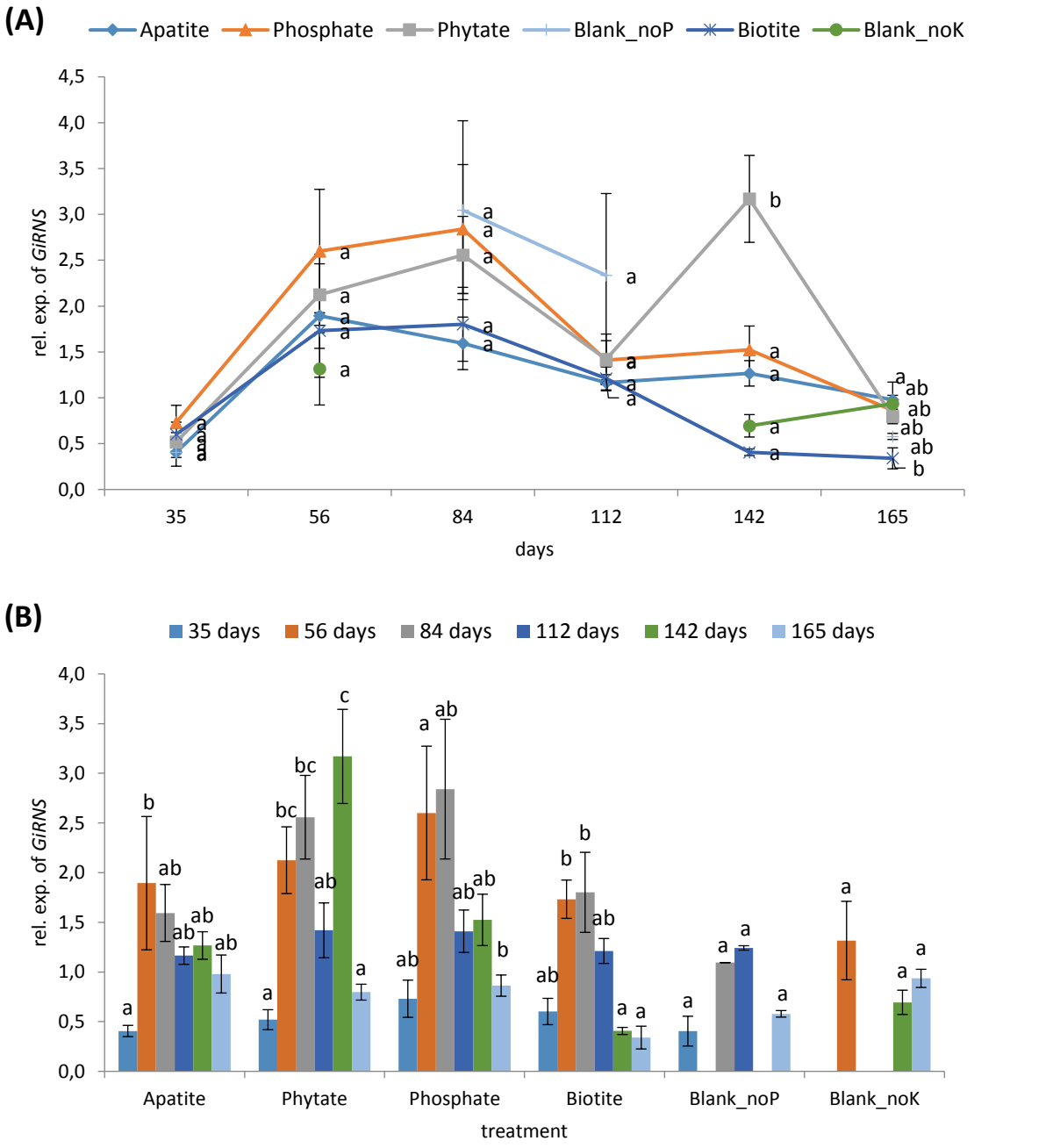

Figure V.7: Relative expression of the fungal marker gene *GiRNS* in the plant roots of the One Arm Experiment. (A) shows the differences in gene expression between the treatments for each harvesting point, (B) shows the differences in gene expression between the harvesting points for each treatment. Plants were harvested after 35, 56, 84, 112, 142 and 165 days in the experimental setup. RNA was extracted from the root material. Relative *GiRNS* transcript levels were determined using RT-qPCR analysis with *LeTefa* as a normalizer. Data are given as mean values +/- SE (n=5; n=3 for blanks). Different letters indicate significant differences (One-Way ANOVA with ad hoc Tukey's Test; $p<0.05$).

5.2.2 Phosphate Transporter Gene Expression in Response to Different Phosphate Sources

The expression of the phosphate transporter *LePT4* shows no clear trend over the time of 112 days (Fig. V.8). Within each harvesting point, the treatments show nearly no significant differences, with the exception of the biotite plants. After 56 days, there is a significant difference in expression between the biotite and blank_noK plants compared to the phytate and apatite plants, with a higher expression in the apatite and phytate plants. For day 142, the apatite plants have a significantly higher expression than the biotite plants. It is noticeable that the expression in all phosphate-fertlized treatments is significantly higher after 142 days. After 165 days, the expression decreases again. For apatite, the expression after 165 days is still significantly higher than for the first four harvestings. The biotite plants also show a higher expression after 142 days, but this is only significant compared to the second harvesting. Variance in the data is relatively high, which leads to a high standard deviation. The expression pattern has no similarity to the expression of the fungal marker gene. After 21 weeks, the expression of LePT4 increases significantly in all treatments, unlike the *GiRNS* expression, which only increases for the phytate treatment. The biotite plants show the lowest expression of LePT4 throughout the whole experiment, with the same general trends, which is the only resemblance to the *GiRNS* expression.

The expression of *LePT3, too,* shows no statistically significant differences throughout the whole experiment, with the exception of a higher expression in the apatite plants, compared to the biotite plants after 142 days. Although the differences are otherwise not significant, the biotite plants again have the lowest expression. There were no statistically significant differences between the harvesting points within each treatment.

There is also no correlation between the *LePT4* expression and the P gained in the experiment. A higher amount of gained P is not necessarily linked to a higher *LePT4* expression. The same applies for *LePT3*. In addition, there was also no correlation between the analyzed transporter genes and the CO_2 respiration per mol of organic carbon, the total microbial biomass or the fungal or bacterial PLFA markers.

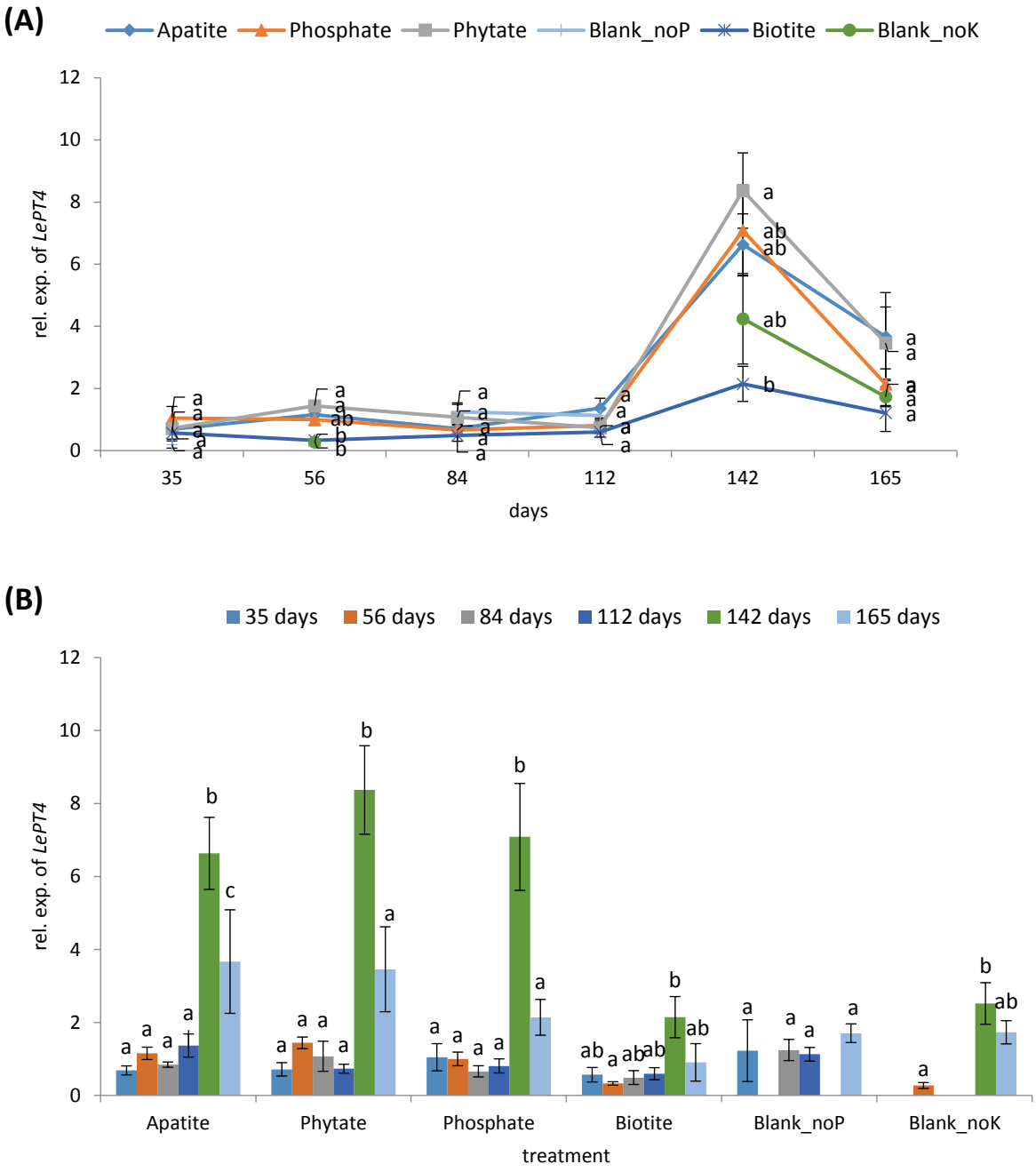

Figure V.8: Relative expression of the phosphate transporter *LePT4* in the plant roots of the One Arm Experiment. (A) shows the differences in gene expression between the treatments for each harvesting point, (B) shows the differences in gene expression between the harvesting points for each treatment. Plants were harvested after 35, 56, 84, 112, 142 and 165 days in the experimental setup. RNA was extracted from the root material. Relative *LePT4* transcript levels were determined using RT-qPCR analysis with *LeTefa* as a normalizer. Data are given as mean values +/- SE (n=5; n=3 for blanks). Different letters indicate significant differences (One-Way ANOVA with ad hoc Tukey's Test; $p<0.05$).

5.2.3 Sugar Transporter Gene Expression in Response to Different Phosphate Sources

The expression of sugar transporter *SlSUT2* is less variable than for the phosphate transporters (Fig. V.9). There are no significant differences between the treatments for each harvesting point with the exception of a lower expression of the blank_noK plants after 56 days. There are also no significant differences within the treatments between each harvesting point. Only the plants treated with apatite have a significantly higher expression after 56 days, compared to the last three harvesting points. The first harvesting point is also significantly higher than harvesting points four and six. Although there are no more significant differences, there is a general trend towards a higher expression in the first weeks of the experiment. The plants treated with biotite again show the lowest expression in all treatments, except for day 112. The variance in each group is very high, which leads to high standard deviations, which partly explains the absence of statistical differences.

Same as for the Phosphate transporter expression, there was no correlation between *SlSUT2* expression and P gained in the experiment, CO_2 respiration per mol of organic carbon, the total microbial biomass or the fungal or bacterial PLFA markers.

There is, however, a correlation between the *SlSUT2* expression and the time spent in the experiment. After 112 days, the expression decreased significantly and stayed low until the end of the experiment.

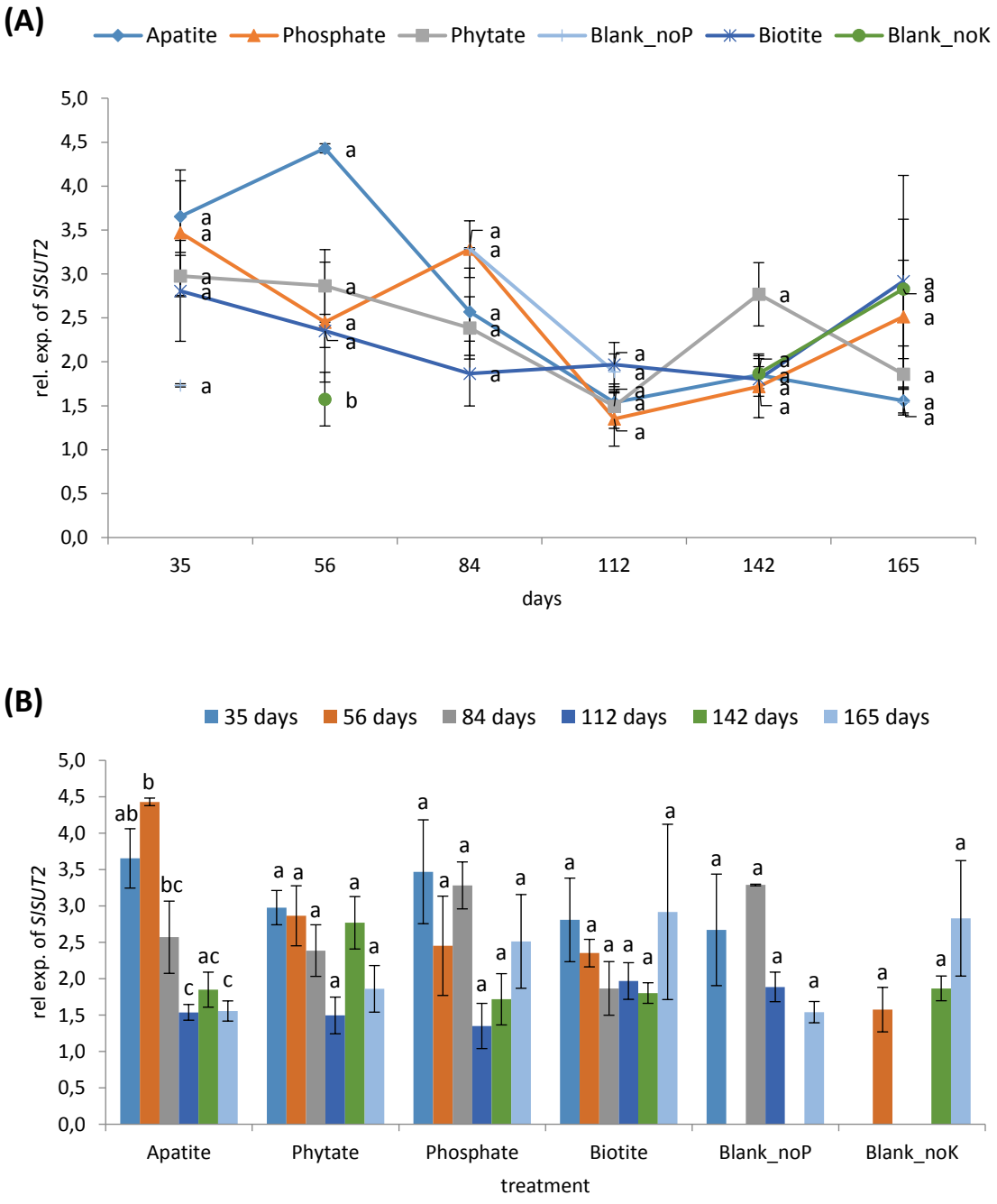

Figure V.9: Relative expression of the sugar transporter *SlSUT2* in the plant roots of the One Arm Experiment. (A) shows the differences in gene expression between the treatments for each harvesting point, (B) shows the differences in gene expression between the harvesting points for each treatment. Plants were harvested after 35, 56, 84, 112, 142 and 165 days in the experimental setup. RNA was extracted from the root material. Relative *SlSUT2* transcript levels were determined using RT-qPCR analysis with *LeTefa* as a normalizer. Data are given as mean values +/- SE (n=5; n=3 for blanks). Different letters indicate significant differences (One-Way ANOVA with ad hoc Tukey's Test; $p<0.05$).

6. Discussion of the One Arm Experiment

The One Arm Experiment addressed the question if the mycorrhizal plant controls the amount of carbon invested in the fungus, depending on the accessibility of the phosphate source available to the fungus. The hypothesis was that the plant changes the carbon investment over time, depending on the accessibility of the P source. Furthermore, I wanted to determine if the expression pattern of the major phosphate transporter *LePT4* as well as the sugar transporter *SlSUT2* changes due to the accessibility of the P source or the change in plant carbon investment. In that case, the gene could function as a marker for phosphate or carbon trading.

6.1 Carbon Investment of the Plant in Response to Different Phosphate Sources

The experiment showed that the fungus was able to use each form of phosphate. The plants have been supplied with similar amounts of P after 112 days in the experiment, regardless to the treatment the plants received. Therefore, it is safe to say that phytate and apatite can be utilized by the fungus for provision of P. This is in accordance with former studies suggesting that AM fungi are able to use organic substances as a phosphate source (Koide & Kabir 2000; Joner et al. 2000; Feng et al. 2003; Wang et al. 2017; Wang et al. 2013). Furthermore, the data from the One Arm Experiment supports the theory that AM fungi are able to participate in the weathering of minerals containing P, like apatite (Koele et al. 2014).

The phosphate from the different sources was gained by the fungus with different kinetics, which affected the carbon investment over time. The plants treated with phosphate maintained a constant investment of carbon into the fungus, which was lower than for the phytate and apatite treated plants. After 56 days, the phytate plants invested 4 to 5 times more carbon into the fungal biomass to gain the same amount of phosphate than the phosphate plants. The apatite plants also invested 4.5 times more carbon than the phosphate plants, but only after 84 days. The peak in carbon investment for each treatment represented by the fungal biomass was always preceded by a peak in gained P. The different kinetics between the sources suggests different uptake mechanisms of the fungi during the P acquisition, depending on the accessibility of the source. The acquisition of P from apatite seems to take more time than from phytate or the phosphate solution.

6.1.1 Phosphate Acquisition from Organic Compounds

Fungal P acquisition from organic compounds such as phytate has been the focus of several studies, but its mechanism remains unclear. As discussed in the introduction, one theory is that the AM fungi profit from other microorganism in the soil, which hydrolyse organic compounds, such as soilborne bacteria. It has been shown that AM fungi can influence the growth and behavior of other microorganisms, especially phytase-producing bacteria (Linderman 1992; Fitter & Garbaye 1994; Toljander et al. 2007; Zhang et al. 2014; Zhang et al. 2016). The second theory is a direct hydrolysation of organic compounds by the AM fungi without the aid of other organisms. It was suggested that the fungus can produce and excrete hydrolyzing enzymes such as acid phosphatase (Wang et al. 2013; Zhang et al. 2014; Saito 1995; Joner et al. 2000; Tarafdar & Marschner 1994; Joner & Johansen 2000; Koide & Kabir 2000) or phytase (Wang et al. 2017). The One Arm Experiment supports the theory of direct AM fungal P acquisition from phytate, although the possibility of PSB in the outer chamber of the set-up cannot be entirely excluded. In contrast, the PLFA and NLFA measurement confirms the presence of bacteria in the substrate of the outer chamber, though it is not clear which bacteria are present and if or how much they contribute to phytate hydrolysis. Therefore, the One Arm Experiment would benefit from a detailed analysis of the soil and its phytase and acid phosphatase content, as well as its microbiome. Further experiments should include this analysis for a better understanding of the fungal uptake mechanisms. Either way, the uptake and delivery of phytate-derived phosphate took more time and a higher carbon investment than for inorganic phosphate. An effect of the time spent in the experiment was also observed by Feng et al. (2003). The experiment used a set-up similar to my experiment, where the compartment with the additional P source was only accessible for the fungus. Three different organic P sources including phytate were used in comparison to inorganic phosphate, and the plants were harvested after 5, 7 or 10 weeks in the experiment. A contribution from the hyphal compartment to the plant P nutrition could be observed for all treatments, but it was smaller for the organic sources than for the inorganic P sources for the first two harvests. For the third harvest, though, the contribution from the organic sources increased and was similar or greater (for phytate) than from the inorganic source. The carbon investment of the plant was not investigated in this study. The kinetic observed in the One Arm Experiment is in accordance with the findings from Feng et al. (2003). The study suggested that the correlation between the growth stage of the mycorrhizal plant and the increase in bioavailability of the organic phosphate sources can be explained by the increasing effect of extraradical AM hyphae on the activity of acid and alkaline phosphatases in the soil. This effect has been described by several studies (Tarafdar & Marschner 1994; Tarafdar & Marschner 1995; Song et al. 2000; Feng et al. 2002). Though my findings cannot directly confirm this theory, I observed the same kinetic in my experiment, with a higher P contribution from phytate than from the other P sources after 8 weeks.

However, other explanations are also possible, including the uptaking mechanisms discussed above. The fungi need energy and therefore carbon to produce necessary enzymes like phosphatases, which is represented by the higher C investment of the plant. The C investment for each treatment in the One Arm Experiment rose after the cumulative P gained by the plant from this source had its peak. This points to a direct correlation of P gain and carbon investment. Furthermore, an enzymatic uptaking process seems to need more time than a direct uptake. It has been demonstrated that the ability of AM fungi to utilize inorganic P is up to 8 times greater than for phytate (Koide & Kabir 2000). In the experiment, similar amounts of P have been transported in the same time span from either a relatively small amount of inorganic P (35 µM P) or an 8 times higher amount of phytate P (280 µM P). If a higher amount of phytate is necessary to gain the same amount of P in the same time than from an inorganic P source, it seems quite possible that it takes longer for the fungus to gain the same amount of P from phytate as from the inorganic source if the initial P supply was the same. However, the experiment conducted by Koide & Kabir (2000) was analyzed after 113 days, with no harvests until the end of the experiment. Therefore, the kinetic throughout the experiment could not be observed. My experiment is comparable considering the overall length, but the time points in between were analyzed, too. At the end, my plants also accumulated nearly the same amount of P from both sources, though the experiment started with the same initial amount of P in each source. The phytate treated plants gained more P after 56 days, whereas the phosphate treated plants reached a peak after 84 days. This speaks against the theory of a different ability to utilize the two P sources or at least against the extent of it proposed by the study. Though more carbon investment is needed, the utilization of P from phytate does not take extensively more time. An explanation for the uptake pattern in both experiments could be that the fungus just takes as much phosphate as needed at the time, even though more phosphate is present. It is also possible that only a certain amount of phosphate can be taken up and transported at once, and both sources covered this maximal amount. Another explanation for the increased carbon investment could be an enhancement in hyphal growth and branching, in order to explore a greater volume of substrate for easier P acquisition. It is likely that each theory can be applied and that more than one factor is responsible for the kinetics in P acquisition.

6.1.2 Phosphate Acquisition from Minerals

Studies about fungal mineral weathering have mostly focused on ectomycorrhizal fungi, and direct nutrient uptake from rock-forming minerals by EMF has been described in various studies (Leyval & Berthelin 1989; Paris et al. 1996; Wallander et al. 1997; Glowa et al. 2003; Leake et al. 2008; Bonneville et al. 2009). However, different experiments also showed the potential of AM fungi in

mineral weathering (Berthelin & Leyval 1982; Sanz-Montero & Rodríguez-Aranda 2012; Arocena et al. 2012; Quirk et al. 2012; Koele et al. 2014). One of the most recent ones used nylon mesh bags with either reactive rock phosphate or crystalline apatite, which were buried in field sites dominated by either EMF or AMF. In both field sites, linear features described as tunnels, as well as fungal hyphae, were found on the mineral grains and soil particles, indicating that both fungal types are contributing in mineral weathering (Koele et al. 2014). The One Arm Experiment gives further evidence for arbuscular mycorrhizal weathering of apatite, as a P gain for the plant could be observed for plants treated with apatite as a phosphate source. It was suggested that the "tunneling" observed by several above mentioned studies on EMF weathering is based on the secretion of organic acids, since they enhance the release of phosphate from apatite and other minerals (Arbel et al. 1991; Welch et al. 2002; Goyne et al. 2006; Sagoe et al. 1998; Nakamaru et al. 2000; Johnson & Loeppert 2006). AM fungi are also able to excrete organic acids (Tawaraya et al. 2006; Toljander et al. 2007), hence it is possible that AMF mineral weathering uses the same mechanism. Saprophytic fungi or acidifying of topsoil through organic matter decomposition are also under discussion as a contribution to tunnel formation (Sverdrup 2009). The surface of the apatite grains were not analyzed after the experiment and therefore, my data cannot contribute to this discussion. Koele et al. (2014) suggests that general mycorrhizosphere acidification is the most likely mechanism for nutrient uptake by AM fungi. However, in their field study, a differentiation between saprophytic or mycorrhizal fungal hyphae was not possible, and saprophytic fungi as well as other microorganisms could not be excluded from the experiment. Although I could not exclude bacteria from the outer chambers either, it is unlikely that a population of saprophytic fungi was able to develop under my experimental conditions, and the analysis of the substrate from the outer chamber confirms this. Therefore, the One Arm Experiment would support the theory of the active secretion of organic acids by the mycorrhizal fungi to make mineral phosphate available to them. A further acidification of the substrate by bacteria, though, cannot be excluded. A more thorough analysis of the sand in the outer chamber with a focus on organic acid extraction could help to further understand uptake mechanisms. Either way, the necessary acidification to make the mineral phosphate accessible to the fungus seems to take several weeks. Either the fungus, or soilborne bacteria, or most likely both have to produce organic acids in order to acidify the substrate. This will take energy in form of carbon, which must be delivered to the fungus by the plant. Furthermore, the fungus needs to grow and infiltrate the substrate more thoroughly to get access to as much apatite grains as possible to maximize the surface for acidification. Hence, even more time and energy is needed. Both explains the enhanced carbon investment of the plant compared to plants fertilized with inorganic phosphate. It is possible that a certain pH is necessary to dissolve the phosphate from the apatite in a sufficient manner, and the accumulation of acids to reach this pH might take several weeks.

6.1.3 Comparing of P Acquisition from Phytate and Apatite

Comparing the kinetics and carbon investment of P uptake from phytate against uptake from apatite in my experiment, both mechanisms seem to need a similar amount of energy. For both treatments, 4-5 times more carbon was invested from the plant to gain the same amount of phosphate than for the inorganic phosphate treatment. This could be either due to an increased hyphal growth in these treatments or because of the need to produce exudates in order to mine the P source. It is likely that both factors are important. Assuming a similar hyphal growth rate for both treatments, the energy needed to produce either phosphatases/phytases or organic acids also seems to be in a similar range. The process leading to the release of P from apatite is more time-consuming than for phytate, as more time was needed to gain the same amount of P. This would support the theories about P uptake from both substances described above. As mentioned before, it is possible that a certain pH is needed in order to dissolve P from a mineral. Therefore, the excreted organic acids would have to accumulate. Due to diffusion in the substrate, more organic acid has to be produced and excreted to maintain the localized low pH value. In contrast, the hydrolysis of a substance with the aid of enzymes should be quite direct and not as time consuming. As soon as an enzyme is exuded, it will work on its substrate. Still, this process needs more energy than a direct uptake of P, as has been discussed above. Enzymes have a half-value period and therefore, they have to be produced frequently again. Furthermore, they need to be produced an excreted at each site with phytate contact, same as the organic acids for apatite weathering. These two different uptake mechanisms would explain the different kinetics with similar carbon investment.

6.2 Transporter Gene Expression in Response to Different Phosphate Sources

6.2.1 Mycorrhizal Abundance as Determined via Fungal Marker Gene Expression

I wanted to determine if the mycorrhiza induced transporters *LePT4* and *SlSUT2* in tomato could work as a potential marker for either the phosphate or the sugar transfer in the symbiosis. Furthermore, the experiment was an opportunity to check the often observed correlation between fungal abundance and *LePT4* expression, which is based on the exclusive expression of these transporters in arbusculated cells (Javot, Penmetsa, et al. 2007; Harrison et al. 2002; Nagy et al. 2005; Nagy et al. 2009). Therefore, I also measured the RNA expression of the fungal marker gene *GiRNS*. From the first to the second harvest, the abundance of the fungal genes in the tomato roots increased in all treatments, and it decreased again for the fourth harvest. For the fifth and sixth harvest, *GiRNS* expression stayed the same for apatite and phosphate, it decreased further for biotite, and increased significantly for phytate before decreasing again to the same level as the other

phosphate treatments. I cannot compare the *GiRNS* expression with other methods to characterize the mycorrhizal colonization of the roots, since no other methods came to use in the experiment. However, the data from the first four harvests can be compared to the fungal 16:1ω5c PLFA marker in the outer compartment. The values were low for the first two harvests for all treatments, which is in accordance with the *GiRNS* expression. A relatively low mycorrhiaztion of the roots could lead to a low abundance of fungal PLFA in the outer chamber. For the second harvest, the *GiRNS* expression increased, whereas the PLFA marker stayed low. A possible explanation would be that the fungal growth in the outer compartment took longer to develop than the root colonization, since the outer chamber is more difficult to reach for the fungus. At the third harvest, the PLFA marker in the phytate treated plants increased, while the other two treatments stayed the same, with the apatite plants showing the lowest values. For the *GiRNS* expression, the apatite plants also had the lowest abundance between the treatments, though the differences were not significant. Interesting is the fourth harvest, where the *GiRNS* expression decreased, while the PLFA marker abundance went up for the apatite plants. Moreover, the PLFA marker stayed relatively low for the phosphate treated plants, whereas the *GiRNS* expression increased for harvest 2 and 3. Aside from the overall low values for both markers for the first harvest, the *GiRNS* marker abundance does not correlate with the PLFA abundance in the outer chamber. This stands against findings from Olsson et al. (1997), where the extraradical/intraradical biomass ratio was preponderantly not influenced by a change in P soil levels, with the exception of a lower extraradical biomass for the highest P treatment. In the One Arm Experiment, the *GiRNS*/PLFA ratio differed noticeably between the harvests and within each treatment, with the lowest ratios in general throughout the experiment for the phosphate treated plants. However, different measurement techniques for the IRM and ERM were used, which makes it difficult to compare the results. Still, the low ratios for the phosphate treated plants are in accordance with other research, showing that a high phosphate supply in the soil can lead to a decrease in extraradical hyphal growth (Abbott et al. 1984; de Miranda & Harris 1994b; de Miranda & Harris 1994a), including the aforementioned study (Olsson et al. 1997). Apparently, the internal and external hyphal growth in a plant system does not depend directly on one another and an intensive extraradical growth is possible with a high or a low degree of mycorrhiaztion in the roots. The same applies for a low growth rate of extraradical mycelium, though both can be influenced by external factors, as the cited studies show. Furthermore, the phosphate source seems to have no influence on the degree of root colonization, since there are no statistical differences in *GiRNS* expression between the treatments, with the exception of the high expression in the phytate plants in the fifth harvest. Even the control plants receiving no phsophate at all showed no differences in *GiRNS* expression, although the plants showed no gain in P over the time and the PLFA marker in the outer chamber was exceptionally low.

6.2.2 Influence of the Mycorrhization on *LePT4* Expression

The expression of the phosphate transporter *LePT4* showed little changes throughout the first 112 days of the experiment and was relatively low in all treatments. The only significant differences between the treatments were a lower expression for the biotite and the control plants after 56 days. Hence, *LePT4* expression does not correlate with the expression of the fungal marker gene. For example, *GiRNS* expression increased for the second harvest, whereas *LePT4* expression stayed low until the sixth harvest. There were harvests throughout the experiment, where the expression was low for both genes, while other harvests had a high expression for one of the genes and a low expression for the other. One similarity is the relatively high expression in the phytate treated plants for day 142 for both genes. The only plants which show a slight similarity in their gene expression throughout the whole experiment are the biotite treated plants with relatively low values for both genes compared to the other treatments. The low *LePT4* and *GiRNS* expression in the biotite treated plants can both be explained by their regular fertilization with phosphate. The plants could take up P through the direct P pathway and the elevated induction of mycorrhizal phosphate transporters was not necessary to maintain a healthy P status. A sufficient amount of P in the medium and therefore the plant roots leads to a decrease in mycorrhization as well as *LePT4* expression, as has been shown before (Menge et al. 1978; Thomson et al. 1986; Schmidt et al. 2010; Breuillin et al. 2010; Bonneau et al. 2013; Nagy et al. 2009). This link could lead to the assumption that the degree of mycorrhization indeed correlates with *LePT4* expression (Nagy et al. 2009; Breuillin et al. 2010). However, the obviously different expression patterns of *LePT4* and *GiRNS* for the other treatments strongly speak against this theory. This further supports my findings from the split root experiment. The split root system showed a correlation between *LePT4* expression and the fungal marker gene, but the degree of mycorrhization was not sufficient to explain the differences in *LePT4* expression (see section 4.1.2). I proposed that other factors, as the P availability, must play a role in altering gene expression. Though the suggestion has been made to use the expression of mycorrhizal phosphate transporters as a marker for a functional symbiosis (Javot, Pumplin, et al. 2007), my data shows that this approach can be misleading and only applies under certain conditions.

6.2.3 Influence of Phosphate Availability on *LePT4* Expression

My second hypothesis tested in both experiments was, that the source of P influences *LePT4* expression. However, with the exceptions of the biotite and the “no K” control plants, there were no significant differences between the treatments in *LePT4* expression. The expression pattern of each treatment follows the same trend. Therefore, it is safe to say that the phosphate source has no influence on *LePT4* expression, either. The amount of energy, which has to be invested to gain P from

a nutrient source is not mirrored in the P transporter expression. Hence, I have to refuse my initial hypothesis. This was not clear after the split root experiment, since the apatite treated plants showed a relatively low *LePT4* expression compared to the phosphate treated plants. The One Arm Experiment shows that the weathering of apatite took at least 84 days and after 6 weeks, just a little amount of P could be gained. The plants in the split root experiment were harvested after six weeks. Therefore, nearly no apatite weathering will have occurred, which would explain the relatively low *LePT4* expression. The question if the P source has an influence on *LePT4* expression, provided that the same amount of P is gained from the source, could therefore not be answered sufficiently in the split root experiment. The difference between the phosphate treatment and the apatite treatment, which occurred in the split root design, but not in the one arm design, can likely be explained by this delay in P gain through apatite weathering as well as by the different set-ups, where the phosphate sources were either directly available for both symbiotic partners (split root) or just for the fungus (One Arm), after a certain distance was covered by hyphal growth.

In the split root experiment, I found that *LePT4* expression correlated with the P supply in the soil. If no P was available, the *LePT4* expression was lower compared to a minimum supply with P. In the One Arm experiment, all plants received a similar supply of P, just the P source differed. Since my initial hypothesis proved to be wrong, it could be expected that the phosphate transporter expression was similar between the treatments. The data from the One Arm experiment therefore further supports the findings already made in the split root experiment. Not the source of P is of importance, but the amount of P available to the fungus, and hence the plant roots. All plants were able to gain P with the help of the mycorrhizal fungi. The amount of P changed in between the harvests, but after 4 harvests, a similar amount of P was gained for each treatment. Ostensibly, small differences in P availability seem to have no significant impact on *LePT4* expression in my experiments. Changes in expression occur if nearly no phosphate is available (as seen in the split root experiment) or if the P availability for the plant is high enough and the plant does not depend on the fungus. The latter has been described in the literature (Breuillin et al. 2010; Nagy et al. 2009) and is also most likely the reason for the lower *LePT4* expression in the biotite treated plants in the One Arm Experiment.

6.2.4 Influences of Mycorrhization and Phosphate Availability on *LePT3* Expression

The *LePT3* expression was nearly the same in all treatments and at all harvests. The expression is therefore likely not influenced by the source of P available to the fungus nor the P gained by the fungus. The differences in mycorrhization also had no influence on *LePT3* expression in this

experiment, although the transporter is described to be AM induced (Gómez-Ariza et al. 2009; Nagy et al. 2009) and repressed by high phosphate availability (Nagy et al. 2009). It is likely that small differences in mycorrhization and phosphate availability have no or just a minor influence on *LePT3* expression. Though the *GiRNS* expression shows differences in the mycorrhization throughout the experiment, these changes were not very distinct. The amount of phosphate available to the fungus was also not drastically different, as all plants had a phosphate source available to them. The repression of *LePT3* in other experiments was triggered by vast amounts of phosphate in the medium (e. g. 60 mg/kg) (Nagy et al. 2009), which is far more than even my biotite treated plants received via the normal fertilization with P. However, the biotite treated plants showed a tendency towards a lower *LePT3* expression, though the differences were not significant. This is in line with the literature as well as the *LePT4* expression in my experiment, though the changes in expression are too small for *LePT3* to function as a reliable marker for P gain through the mycorrhizal uptake pathway. This experiment therefore shows that both phosphate transporter genes, *LePT4* and *LePT3*, cannot function as a marker for the degree of mycorrhization nor for the actual P gain of the plant through the fungus. As mentioned above, the P flux in the mycorrhizal system has to be measured or calculated by other methods and cannot just be correlated with *PT4* or *PT3* expression.

6.2.5 *SlSUT2* Expression in Response to Different Phosphate Sources

The expression of the sugar transporter *SlSUT2* showed nearly no significant differences at all, neither between the treatments nor between the different harvests. I initially hypothesized that *SlSUT2* expression would change according to the phosphate availability in the soil and would be down-regulated if no phosphate was available, since no phosphate would be delivered to the plant. This could not be confirmed in the split root experiment (see section 4.1.4 and 4.2.4), and the One Arm experiment does not support this theory, either. *SlSUT2* expression did not change significantly if P was more easily available for the fungus nor if it was not present at all, as was the case in the control plants. The only significant difference between the treatments was a lower expression in the no K control plants for day 56, and these plants were directly fertilized with phosphate. The biotite plants, which were also fertilized with phosphate, showed a tendency towards a lower *SlSUT2* expression, though not significantly. This could also be seen for the *LePT4* expression, and it seems likely that a preferential use of the direct P pathway instead of the mycorrhizal P uptake pathway leads to the decrease in gene expression, which could also be a possible explanation for the *SlSUT2* expression. The second hypothesis was that *SlSUT2* expression could be used as a marker for the C supply from the plant to the fungus. According to my data, this seems also unlikely. I was able to see differences in carbon investment and P gain throughout the experiment. However, the *SlSUT2*

expression shows nearly no differences between the harvests. There were only significant differences within the apatite treated plants. They had a higher *SlSUT2* expression for the first two harvests compared to the last three harvests. There is a general trend towards a higher expression in the first two to three harvests compared to the last three harvests, though the sixth harvest shows an upward trend again. However, as mentioned before, these differences are not statistically significant. Still, this trend and the differences for the apatite treated plants lead to the impression that sugar is given readily to the fungus in the beginning of the mycorrhizal symbiosis. Sugar is needed by the fungus for hyphal growth as well as the production of exudates. A higher expression at a time where the fungus needs to grow and explore the surrounding soil to provide the plant with nutrients is therefore a logical consequence. However, as discussed in section 4.2.4, it was also suggested that SlSUT2 is responsible for transporting sugar from the periarbuscular space back into the cytoplasm (Bitterlich et al. 2014). The protein is located in the periarbsucular membrane, and an experimentally reduced *SlSUT2* expression led to an increased mycorrhization, without the normally accompanied positive mycorrhizal effect (Bitterlich et al. 2014). With this explanation, the higher *SlSUT2* expression in the beginning of the experiment would lead to a reduced carbohydrate supply from the plant to the fungus. Throughout the experiment, the C supply would increase as the *SlSUT2* expression decreases. This would correlate with the P gain by the plant through the fungal hyphae. Especially for the apatite treated plants, where changes in *SlSUT2* expression were significant between the harvests, this model fits quite well. The relatively low P supply by the fungus in the beginning of the experiment would lead to a upregulation of *SlSUT2* and therefore less carbohydrates for the fungus. With increasing P gain, the *SlSUT2* expression decreases and more carbohydrates would be given to the fungus. Hence, my data supports the theory by Bitterlich et al. (2014), though it cannot directly prove it. Against this theory stands the carbon investment observed in the One Arm Experiment. The apatite treated plants had an overall higher carbon investment than for example the phosphate treated plants. However, most of the carbon was invested after 84 days, where *SlSUT2* expression was decreasing. Furthermore, it is unlikely that changing *SlSUT2* expression is the only way for the plant to regulate carbon transfer to the fungus. The regulation process of SlSUT2 can also be post-transcriptional or post-translational, and in addition, other sugar transporters or proteins are supposedly involved in the carbon trading process. The regulating processes in the mycorrhizal symbiosis proved to be rather complex and further research should not only focus on single genes and proteins, but rather on the whole picture involving various proteins and their interaction.

7. Results of the Split Root Experiment with *Medicago truncatula*

The Split Root Experiment was repeated three times with *Medicago truncatula* instead of tomato. I wanted to determine if the results in RNA expression of mycorrhizal transporter genes in the plant and the fungus changes depending on the plant species involved in the symbiosis. The first repetition was done in the same experimental design as with *Solanum lycopersicum*, including apatite as a phosphate source.

In the second repetition, I changed the design of the experiment, so that only the fungus had access to the phosphate source. This change was made to exclude the possibility of a direct Pi uptake from the plant and to ensure that all P gained by the plant would come from the fungus.

In the third repetition, I additionally labeled the plants with $^{13}CO_2$ to see if the distribution of carbon is affected by the phosphate source available for the fungus. In the labeled plants, PLFA and NLFA measurements were done with the mycorrhized roots in addition to the RNA extraction and measurement of the nutritional plant status.

For all three repetitions, the plants were harvested after six weeks in the experimental design, and root and shoot material was collected for analysis. Root and shoot fresh weight was taken before both sections were frozen and ground in liquid nitrogen for storage.

7.1 First Repetition with Phosphate Fertilization of Roots and Hyphae

7.1.1 Physiological Changes in Response to Different Phosphate Fertilization

In the first repetition, the AN and the PP plants showed a tendency towards a higher shoot weight than the AA, AP, and PN plants, but the difference was not significant. The root weight also showed no significant differences, although there was a tendency towards a lower root weight in the N side of the PN plants compared with the P side (Fig. VII.1).

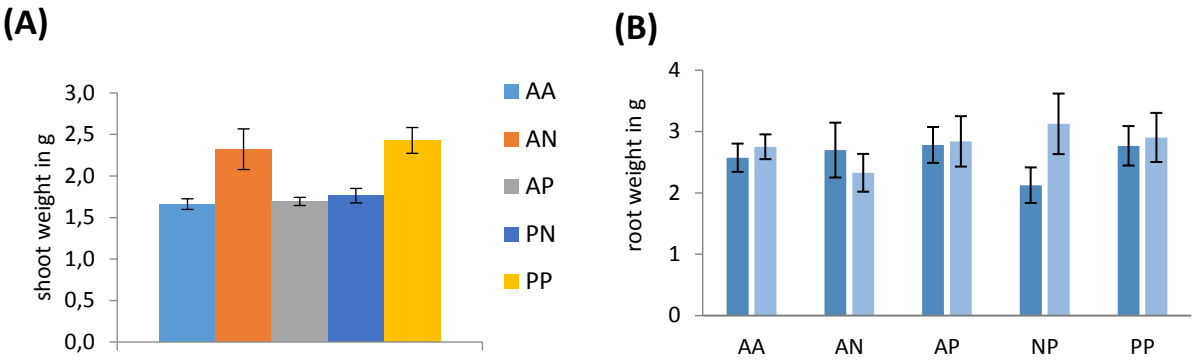

Figure VII.1: Shoot (A) and root (B) fresh weight from the first split root experiment with *M. truncatula*. Plants were kept for six week in the experimental set-up, fresh weight was taken directly after harvesting. PP: plants receiving phosphate on both sides; PA: plants receiving phosphate (P) on one and apatite (A) on the other side; PN: plants receiving phosphate (P) on one and no phosphate (N) on the other side; AA: plants receiving apatite on both sides; AN: plants receiving apatite (A) on one and no phosphate (N) on the other side. (B): Left bars: side with the first letter of the combination; right bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

The mycorrhizal status of the roots was determined via AlexaFluor® staining and microscopy techniques. The mycorrhization was between 86% and 100% in all plants with the lowest amount in the AA plants. Minor differences could be observed between the sides for the AP and the AN plants, but they were not statistically significant (Fig. VII.2).

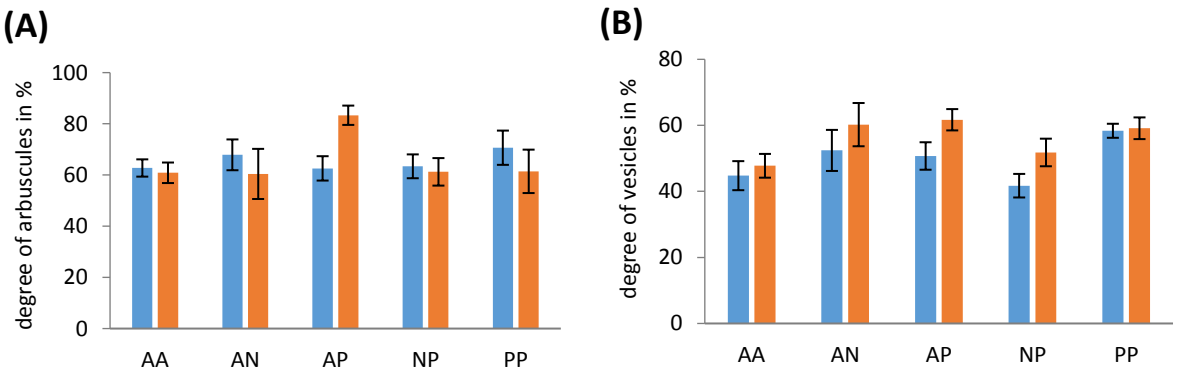

Figure VII.2: Degree of mycorrhization in the roots of the first split root experiment with *M. truncatula.* (A) shows the percentage of arbuscules, (B) the percentage of vesicles. Mycorrhizal status was determine via Alexa staining and microscopy. Blue bars: side with the first letter of the combination; orange bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

The phosphate status of both roots and shoots were analyzed and a CN analysis was performed with the dried shoot material (Fig. VII.3A, B). For the root phosphate status, there are slight differences between the side of the AN and AP plants with a lower phosphate content on the apatite side (Fig. VII.3A). In the shoot material, no significant differences could be observed for phosphate, carbon and nitrogen content (Fig. VII.3B, C, D), though he AN plants showed a tendency towards a lower C content than the others plants.

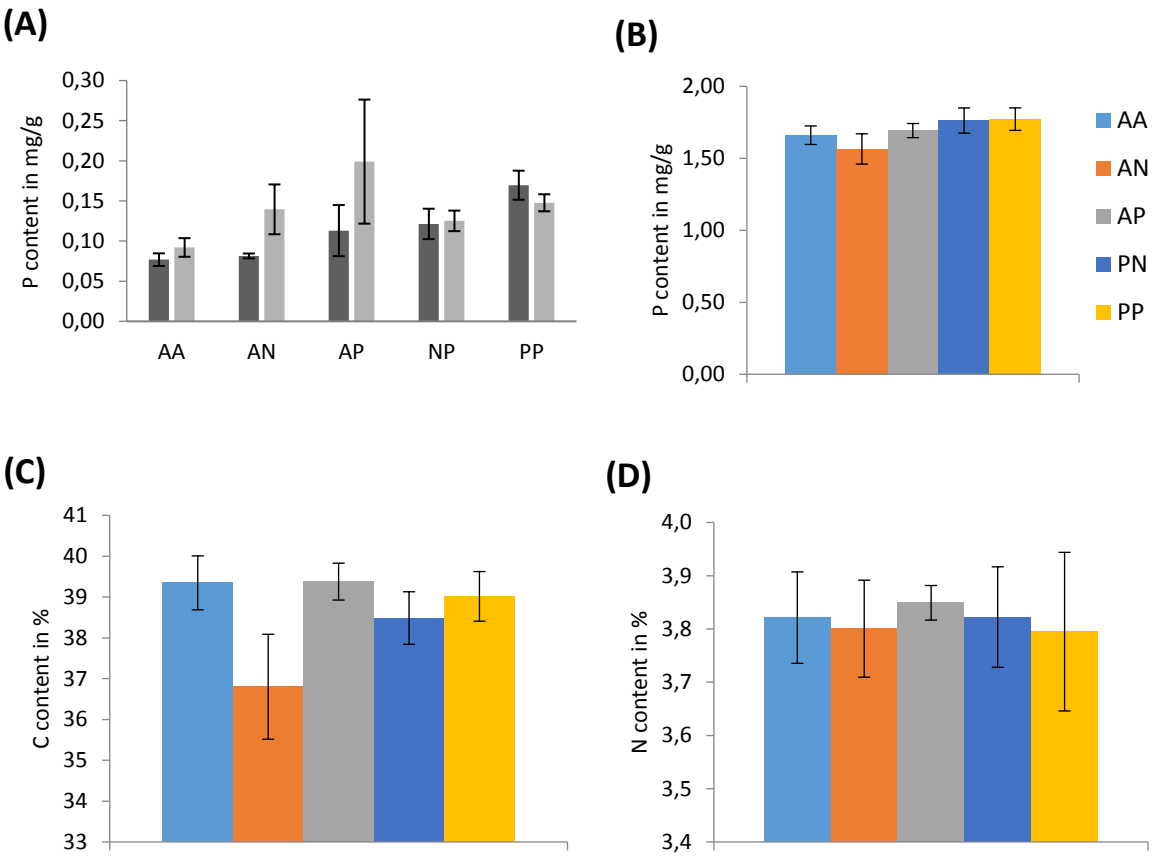

Fig VII.3: Phosphate status of the roots (A) and shoots (B) and carbon (C) and nitrogen content (D) of the shoot material from the first split root experiment with *M. truncatula*. P content in the shoots (A) was determined via ICP measurement, to determine the root phosphate content (B), a photometric test was performed. C and N content (C and D) was measured with the CN analyzer. (A): Left bars: side with the first letter of the combination; right bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

7.1.2 Correlation Between *MtPT4* and Fungal Marker Gene Expression

RNA was extracted from the roots and the expression of *MtPT4* and the fungal marker gene *GiTef*α were measured to determine the influence of phosphate availability on their transcript levels (Fig. VII.4). Both genes show a very similar expression with a significantly higher expression in the NP plants compared to most of the other treatments. For *MtPT4*, the N side of the NP plants has a significantly higher expression than all other treatments except the A side of the AN plants, whereas the P side of the NP plants is only significantly higher than the AA plants and the N side of the AN plants. The expression of *GiTef*α is significantly higher in the N side of the NP plants compared to all other treatments. The P side of the NP plants is only significantly higher than the N side of the AN plants, though a tendency towards a higher expression is visible compared to all treatments.

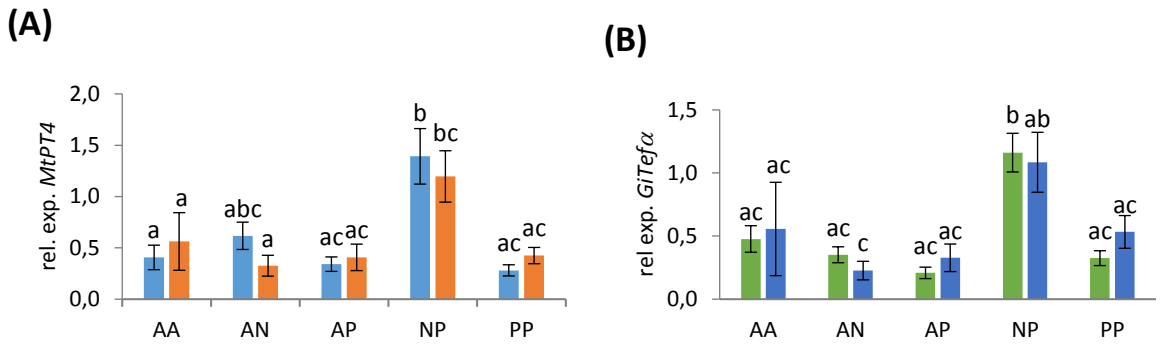

Figure VII.4: Relative expression of *MtPT4* (A) and *GiTef*α (B) in the first split root experiment with *M. truncatula*. RNA expression was measured via RT-qPCR. *MtTef*α was used as a normalizer. Each pair of columns represents a split root system with one column per compartment. Left bars: side with the first letter of the combination; right bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

7.1.3 Fungal Transporter Gene Expression in Response to Different Phosphate Fertilization

To determine if the expression of the fungal genes *GiPT* and *GIMST2* depends on the host species, the RNA expression of these genes in the intraradical mycelium of the roots was measured via qPCR (Fig. VII.5). There is a significant difference between the sides of the AP plants for *GiMST2* expression, with a higher expression on the A side. Furthermore, the A side of the AP plants as well as both sides of the AN plants show significantly higher transcript levels than the AA plants and the P side of the PN plants. For *GiPT*, the expression in the A side of the AP plants is also significantly higher than in the AA plants, the PP plants and the P side of the NP plants. The differences in transcript levels between the treatments is not as pronounced as in the tomato split root experiment.

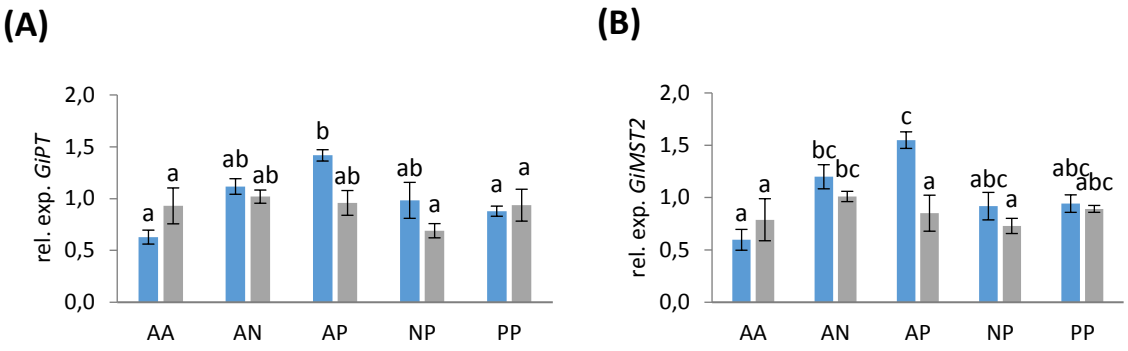

Figure VII.5: Relative expression of *GiPT* (A) and *GiMST2* (B) in the roots of the first split root experiment with *M. truncatula*. RNA expression was measured via RT-qPCR. *GiTef*α was used as a normalizer. Each pair of columns represents a split root system with one column per compartment. Blue bars: side with the first letter of the combination; grey bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

7.2 Second Repetition With Phosphate Fertilization Through the Hyphae

In the second reptition of the experiment, the design was changed, as described above. Due to technical reasons, the PN plants also had to be excluded.

7.2.1 Physiological Changes in the Second Reptition in Response to Apatite Fertilization

After six weeks in the experimental design, the plants were harvested and the phosphate content of the roots and the shoots was measured as well as the nutritional status of the shoot material (Fig VII.6). The P content showed a tendency to be lower in the AA plants, in the root as well as in the shoot material. The difference is significant compared to the PP plants. The C content had a tendency to be lower in the AP plants. It is noticeable that the general nutritional status of the shoots was better in plants receiving more phosphate solution and less apatite. For the nutritional status of Na, Cu, Mn, and Fe, no significant differences between the treatments could be found. For Mg, there was a significant difference between the AA and PP plants with less Mg in the AA plants. There was also significantly less Ca and S in the AA plants compared to the PP and AP plants and less K and Si compared to the PP, AP and AN plants.

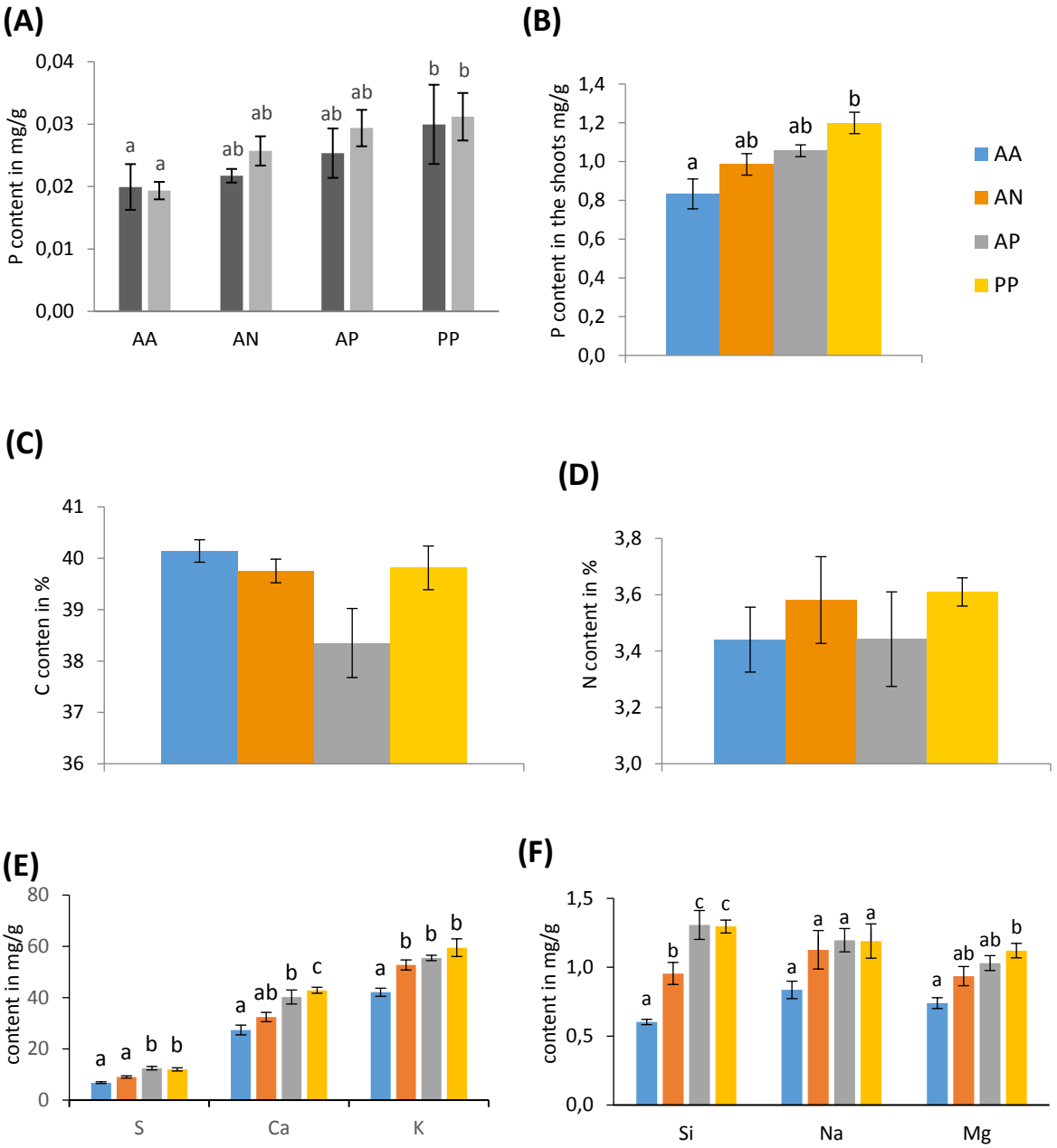

Figure VII.6: Phosphate status of the roots (A) and shoots (B), carbon (C) and nitrogen content (D) and nutritional status (E and F) of the shoot material in the second split root experiment with *M. truncatula*. Nutrient contents in the shoots (B, E and F) were determined via ICP measurement, to determine the root phosphate content (A), a photometric test was performed. C and N content (C and D) was measured with the CN analyzer. (A): Left bars: side with the first letter of the combination; right bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

The degree of mycorrhization was determined via AlexaFluor® staining and microscopy (Fig. VII.7). It was between 94-98% in all plants, with 40-60% of vesicles and 30-50% of arbuscules. No significant differences could be observed within or between the treatments. The AA and AP plants showed a tendency towards a higher degree of arbuscules.

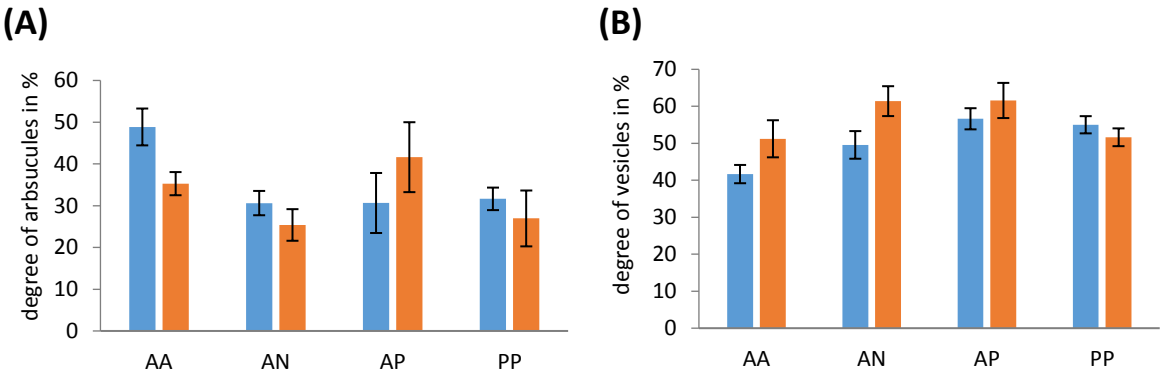

Figure VII.7: Degree of mycorrhization in the roots of the second split root experiment with *M.truncatula*. (A) shows the percentage of arbuscules, (B) the percentage of vesicles. Mycorrhizal status was determine via Alexa stainng and microscopy. Each pair of columns represents a split root system with one column per compartment. Blue bars: side with the first letter of the combination; orange bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

7.2.2 Correlation Between *MtPT4* and Fungal Marker Gene Expression in the Second Repetition

The RNA was extracted from all root samples and the gene expression of *MtPT4* and *GiRNS* was measured (Fig. VII.8). The expression of both genes is very similar with a tendency towards a higher expression in the A side of the AP plants. For *GiRNS*, the A side of the AP plants showed a significantly higher expression than both sides of the AN plants.

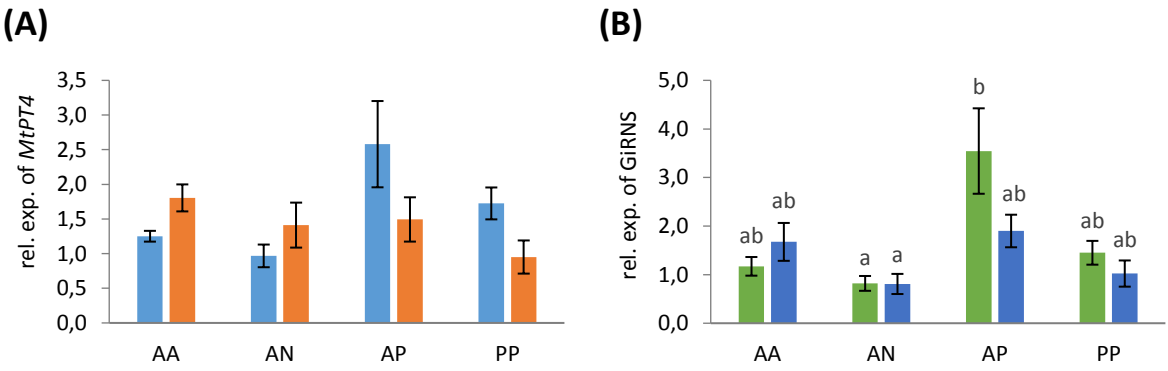

Figure VII.8: Relative expression of *MtPT4* (A) and *GiRNS* (B) in the roots of the second split root experiment with *M. truncatula*. RNA expression was measured via RT-qPCR. *MtTef*α was used as a normalizer. Each pair of columns represents a split root system with one column per compartment. Left bars: side with the first letter of the combination; right bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

7.2.3 Fungal Transporter Gene Expression in the Second Repetition in Response to Apatite Fertilization

The gene expression of the fungal transporter genes *GiPT* and *GiMST2* was measured as well (Fig. VII.9). In this repetition, no significance differences between the treatments or sides could be observed.

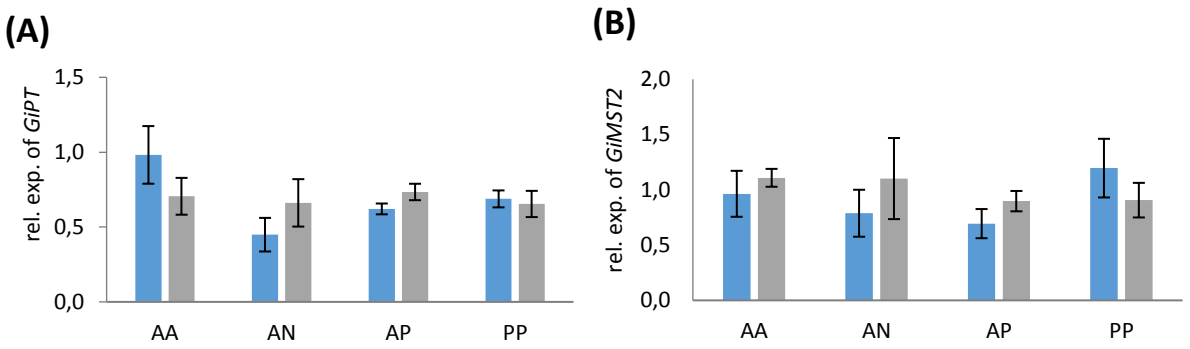

Figure VII.9: Relative expression of *GiPT* (A) and *GiMST2* (B) in the roots of the second split root experiment with *M. truncatula*. RNA expression was measured via RT-qPCR. *GiTef*α was used as a normalizer. Each pair of columns represents a split root system with one column per compartment. Blue bars: side with the first letter of the combination; grey bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

7.3 Third Repetition with Phosphate Fertilization Through the Hyphae and ^{13}C Labeling

The third repetition was done with the same design as the second repetition. The PN plants could be integrated again. For this repetition, a ^{13}C labeling was done 3 days prior to the harvesting.

7.3.1 Physiological Changes in the Third Repetition in Response to Apatite Fertilization

The mycorrhization was measured with AlexaFluor® staining and microscopy (Fig. VII.10). The overall mycorrhization was between 90-98% with a slightly higher mycorrhization for the PP plants, the AP plants and the P side of the PN plants. The degree of arbuscules was between 40-60%, the degree of vesicles between 30-45% and both showed no significant differences. There was a tendency towards a lower mycorrhization and degree of arbuscules on the N side of the NP plants compared to the P side. This tendency cannot be seen for the degree of vesicles.

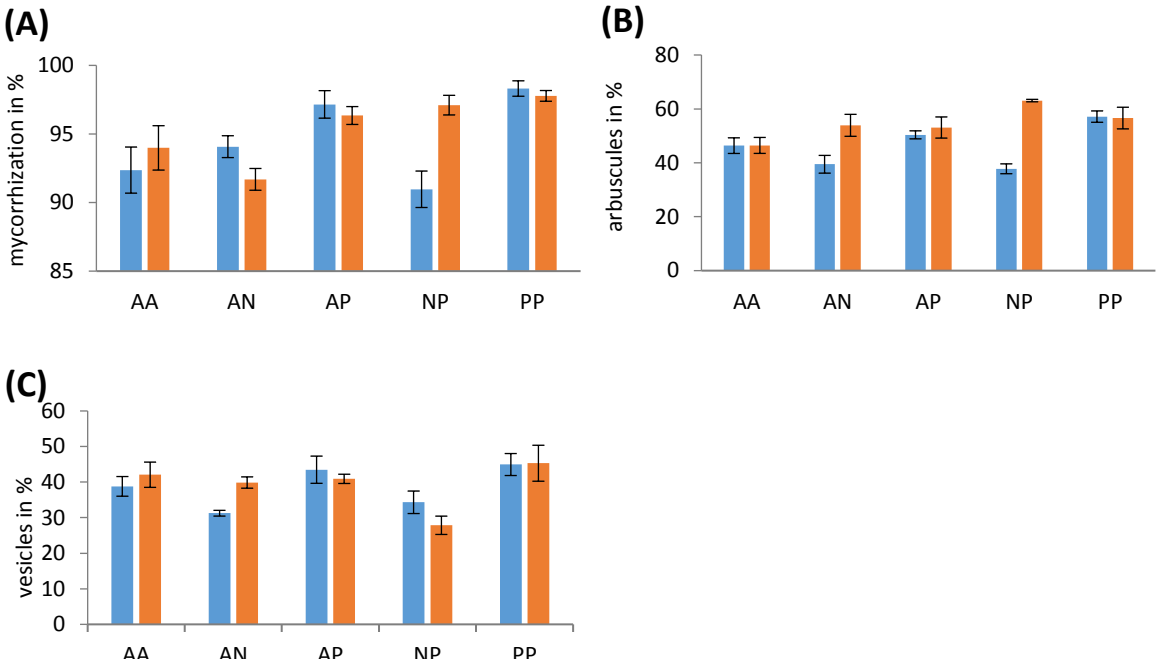

Figure VII.10: Degree of mycorrhization in the roots of the third split root experiment with *M. truncatula*. (A) shows overall mycorrhization, (B) the percentage of arbuscules and (C) the percentage of vesicles. Mycorrhizal status was determined via Alexa staining and microscopy. Each pair of columns represents a split root system with one column per compartment. Blue bars: side with the first letter of the combination; orange bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

The carbon and nitrogen content was measured and no significant differences could be observed, just a slight tendency towards a lower carbon and nitrogen content in the AN plants and a higher C content in the AA plants. The phosphate content of the roots also showed a tendency towards a lower content in the AA and AN plants, which was not statistically significant (Fig. VII.11). The phosphate content of the shoots was higher in the PP plants compared to the AA plants, same as in the second repetition.

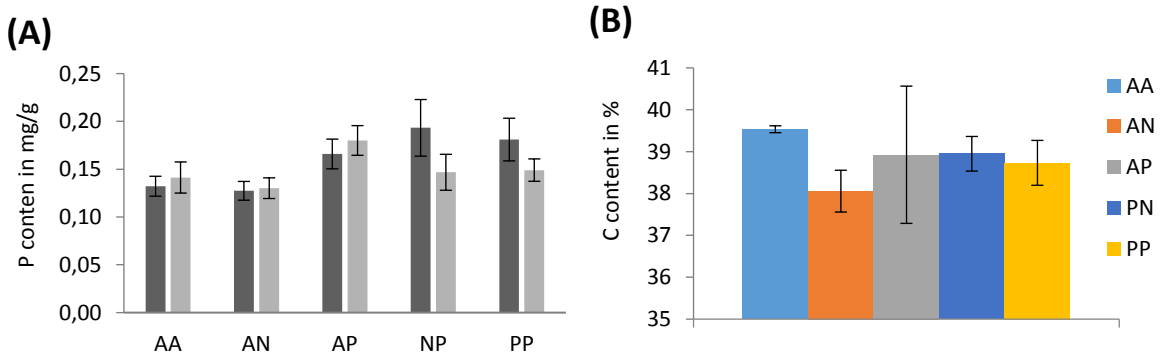

Figure VII.11: Phosphate status of the roots (A) and carbon content of the shoots (B) in the third split root experiment with *M. truncatula*. The P content in the roots was determined with a photometric test. The C content was measured with a CN analyzer. Left bars: side with the first letter of the combination; right bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

7.3.2 Correlation Between *MtPT4* and Fungal Marker Gene Expression in the Third Repetition

The RNA was extracted and the gene expression of *MtPT4* and the fungal marker genes *GiRNS* and *GiTefα* was measured via RT-qPCR (Fig. VII.12). As in the other two repetitions, the expression pattern of the phosphate transporter and the fungal genes was very similar. The PP and the AN plants showed a tendency towards a lower expression, whereas the AA plants and the P side of the NP plants showed a higher expression. Significant differences could be observed for *MtPT4* and *GiTefa* between the PP plants and the P side of the PN plants. For *GiTefa*, there was also a significant difference between the A side of the PA plants and the P side of the PN plants as well as the N side of the AN plants, with a lower expression for the A side. The differences in *GiRNS* expression were not significant.

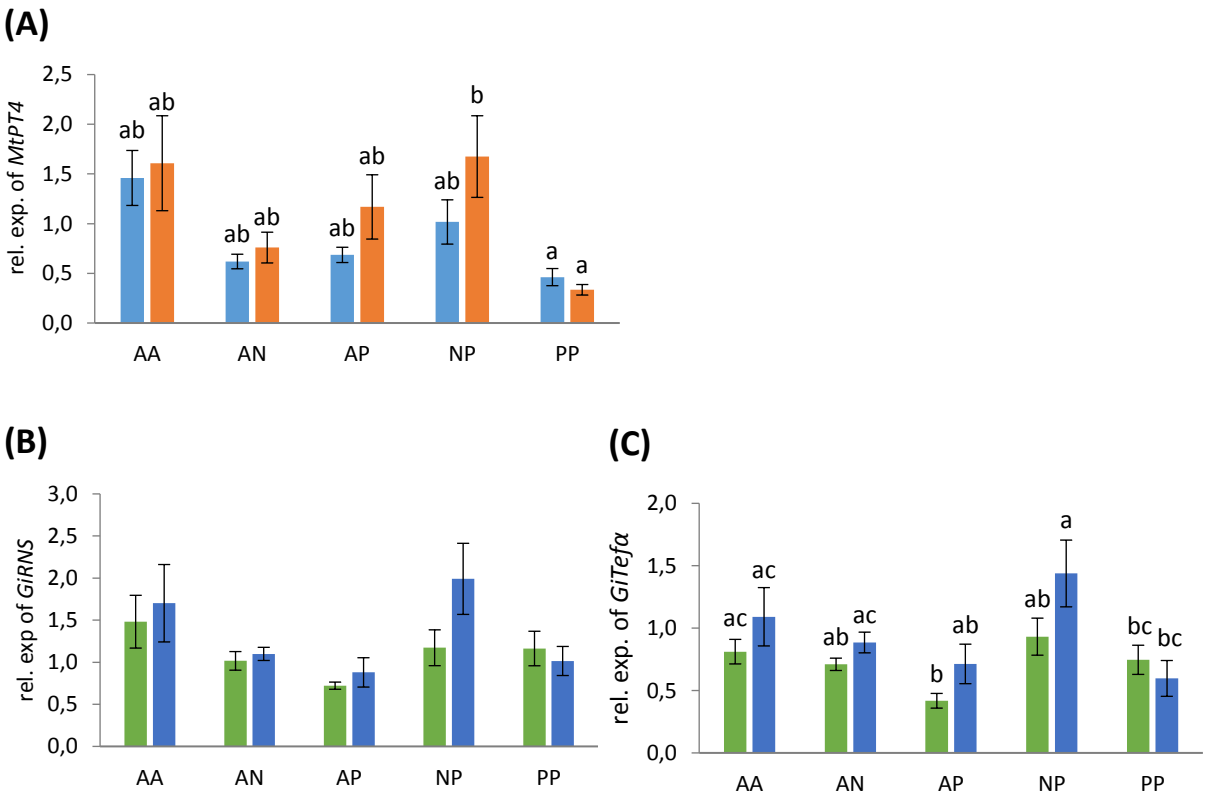

Figure VII.12: Relative expression of *MtPT4* (A), GiRNS (B), and *GiTefα* (C) in the third split root experiment with *M. truncatula*. RNA expression was measured via RT-qPCR. *MtTefα* was used as a normalizer. Each pair of columns represents a split root system with one column per compartment. Left bars: side with the first letter of the combination; right bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

7.3.3 Fungal Transporter Gene Expression in the Third Repetition in Response to Apatite Fertilization

The expression of the fungal transporter genes *GiPT* and *GiMST2* was measured again via RT-qPCR (Fig. VII.13). As seen in the first two repetitions and the tomato split root experiment, the expression pattern of both genes was very similar. The expression on the A side of the AP plants is significantly higher than in the PP plants and the P side of the NP plants. For *GiMST2*, the difference between the sides of the AP treatment is also significant. However, the differences between the treatments are again not as pronounced as in the tomato split root experiment.

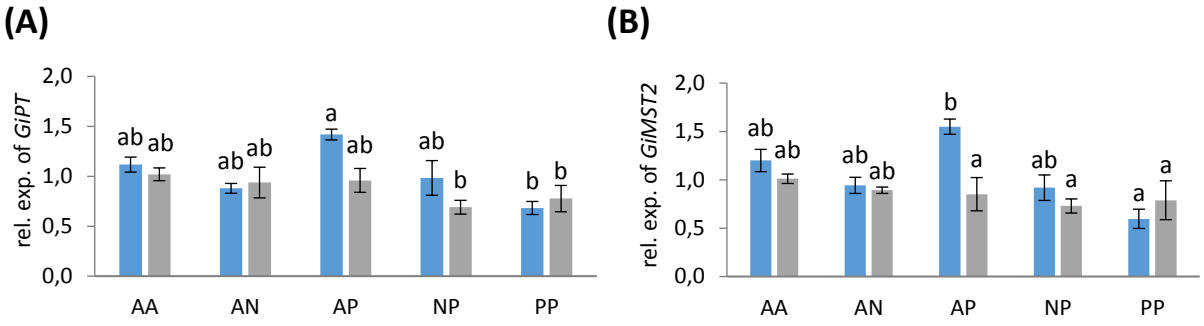

Figure VII.13: Relative expression of *GiPT* (A) and *GiMST2* (B) in the roots of the thirs split root experiment with *M. truncatula*. RNA expression was measured via RT-qPCR. *GiTef*α was used as a normalizer. Each pair of columns represents a split root system with one column per compartment. Blue bars: side with the first letter of the combination; grey bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

7.3.4 Changes in $\delta^{13}C$ and PLFA and NLFA Presence in the Roots in Response to Different Phosphate Accessibilities

The ^{13}C labeling should help to determine if and how the carbon transport from the plant to the fungus is influenced by the P source available to the fungus. For the evaluation of the ^{13}C labeling and the PLFA and NLFA analysis, the bulk $\delta^{13}C$ content in the shoots and roots at the harvesting point was measured in an elemental analysis (Fig. VII.14). There were no significant differences in the $\delta^{13}C$ content of the shoots, and therefore the $\delta^{13}C$ uptake between the treatments. The $\delta^{13}C$ content in the roots showed differences, but they were not significant. There is a tendency towards a lower $\delta^{13}C$ content in the apatite plants, especially for the apatite side of the AP plants.

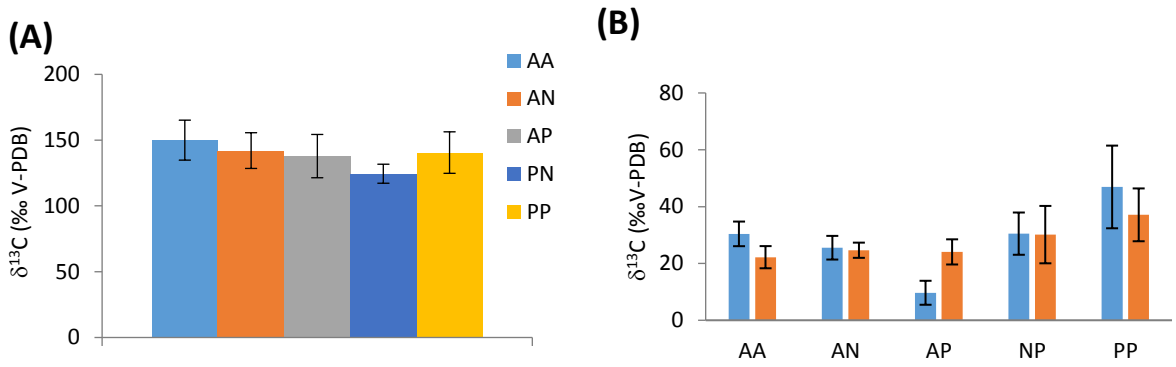

Figure VII.14: $\delta^{13}C$ content of shoots (A) and roots (B) in the third split root experiment with *M. truncatula*. The plants were harvested three days after labeling. Labeling was done for one hour with 1 g of $Ca^{13}CO^3$. Blue bars: side with the first letter of the combination; orange bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

PLFAs and NLFAs were extracted from the labelled plant roots. The amount of the mycorrhizal fatty acid 16:1ω5c in the roots was calculated for the neutral and phospholipid fatty acids. For the PLFA, there was a significant difference between the sides of the NP plants, with a lower amount on the N side. The NLFA was significantly higher on the P side of the AP plants, compared with the A side and compared with the N side of the NP plants. There was also a tendency towards a lower amount in the A side of the AN plants.

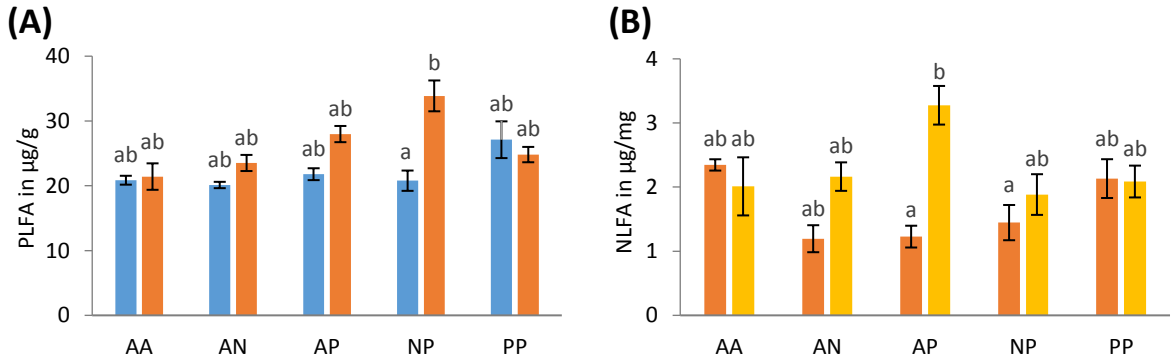

Figure VII.15: 16:1ω5c PLFA (A) and NLFA (B) content of the roots in the third split root experiment with *M. truncatula*. PLFA and NLFAs were extracted from 100 mg ground and frozen root material. Each pair of columns represents a split root system with one column per compartment. Left bars: side with the first letter of the combination; right bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

The amount of ^{13}C incorporated in the fatty acids was calculated on the basis of the $\delta^{13}C$ content of the fatty acids and the roots. The ^{13}C in the 16:1ω5c PLFA represents the amount of plant derived carbon integrated into growing fungal structures such as hyphae or arbuscules. The ^{13}C in the

16:1ω5c NLFA indicates the amount of C integrated into fungal vesicles, their main storage organ. The ^{13}C in the roots divided by the ^{13}C either in the PLFA or NLFA shows how much carbon from the root is given to the fungus. The analysis shows nearly no difference in the ratio of ^{13}C present in the roots to the ^{13}C in the mycorrhizal PLFA 16:1ω5c, which indicates that the P source available to the fungus has no direct influence on the incorporation of plant derived C in the growing fungal structures. The ratios of ^{13}C in the roots to the ^{13}C in the fungal NLFA also show no statistically significant differences. However, there is a tendency towards a lower amount of ^{13}C in the NLFA compared to the root ^{13}C in the AA plants, in comparison with the other treatments. Furthermore, the $\delta^{13}C$ NLFA/$\delta^{13}C$ root ratio differs within the NP and AP treatments, with a lower value on the P side of the AP plants and on the N side of the NP plants. These tendencies could indicate that differences in P supply in different root regions lead to variances in the composition of storage organs.

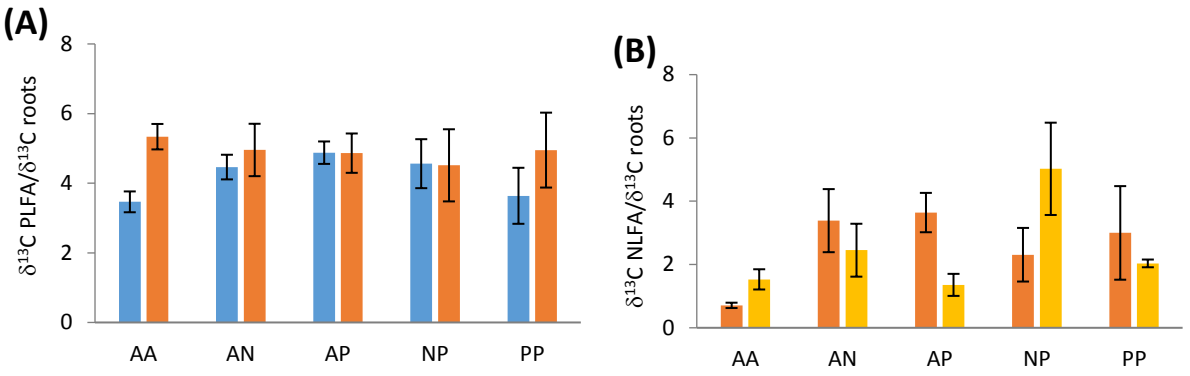

Figure VII.16: $\delta^{13}C$ content of the 16:1ω5c PLFA (A) and NLFA (B) divided by the ^{13}C content of the roots in the third split root experiment with M. truncatula. The plants were harvested three days after labeling. Labeling was done for one hour with 1 g of $Ca^{13}CO^3$. PLFAs and NLFAs were extracted from 100 mg frozen and ground root material. Each pair of columns represents a split root system with one column per compartment. Left bars: side with the first letter of the combination; right bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

8. Discussion of the Split Root Experiment with *Medicago truncatula*

In the three repetitions of the split root experiment with *Medicago truncatula*, I made small changes in the design for two repetitions. A second compartment was added to make sure the phosphate accomplished by the plant was only gained by the fungus. Furthermore, I labeled one of the repetitions with ^{13}C, to get a better understanding of the C transfer from the plant to the fungus.

8.1 Posphate Mining of Apatite by the *Medicago truncatula* Plants

Unlike the tomato plants in the first split root experiment, the *Medicago* plants showed no significant differences in shoot weight between the different treatments. Instead, the plants treated with apatite only had a tendency towards a higher shoot weight. All plants appeared healthy, and no phenotypical differences could be observed for the different treatments. This was the same in all three repetitions, though just the first repetition can really be compared to the tomato experiment, since the experimental set-up was changed for the second and third repetition. Furthermore, there are no differences in the phosphate content of the plants in the first repetition, whereas the tomato plants had significantly less phosphate in the apatite treated plants. This is still mirrored by a tendency towards a lower phosphate content in the AA plants and the A side of the AP and AN plants in the first repetition of the *M.t.* experiment, but nevertheless, the differences were far more pronounced in the tomato plants. In the second repetition, where just the fungus could gain the phosphate, the shoots of the PP plants had significantly more phosphate than those of the AA plants, and the phosphate content of the roots showed the same tendency. A possible explanation for the differences in P acquisition in design 1 between tomato and *Medicago* plants could be that the tomato plants are not able to gain phosphate from apatite on their own, whereas the *Medicago* plants seem to be able to gain at least a bit of phosphate from apatite. It is also possible that they can assist the fungus in mineralizing apatite, if the roots are in close contact with the mineral, for example by exudation of organic acids. Maybe the *Medicago* roots exude more or different organic acids than the tomato roots, which helps to set phosphate free from apatite. Research has shown that the amount and composition of root exudates vary immensely between plant species, and even with the age of the plant or due to environmental factors (Curl & Truelove 1986; Jones 1998). This theory can be supported by studies showing that calcicole plants exude more oragnic acids than calcifuge plants and are more efficient in dissolving mineral P and Fe in lime-rich soils (Ström et al. 1994; Tyler & Ström 1995; Ström 1997; Zohlen & Tyler 2004). The *Medicago* species can often be found on calcerous soils and can be seen as a calcicole plant (Fühner & Runge 2009; M'Sehli et al. 2008). It is therefore adapted to well drained neutral and alkaline soils with a pH around 6-8 and is

intolerant of low soil pH (Evans et al. 1990). It has been shown that *M. truncatula* responds quickly to P-deficiency (Vance et al. 2003). An early study with *Medicago sativa* showed an enhanced exudation of organic acids if the seedlings were under P deficiency stress, suggesting that the exudation of organic acids is enhancing the availability of P for the plant (Lipton et al. 1987). If the expression of phytase and acid phosphatase was genetically enhanced in *Medicago sativa*, the ability to take up phosphate from natural soils might have increased even more (Ma et al. 2012). Tomato cannot be labelled a calcifuge plant, since it has relatively high intracellular calcium levels (Loneragan et al. 1968; Loneragan & Snowball 1969; White & Broadley 2003), but it prefers a slightly acidic soil pH from 5.5 to 6, which is more typical for calcifuge than calcicole plants. However, it seems to be not as efficient in dissolving P from minerals as *M. truncatula*. It is therefore possible that it exudes less organic acids. Various studies seem to support this theory. In tomato, the amount of carboxylic acid in root exudates did not increase in response to P-deficiency, whereas it did increase in chickpea and white lupin (Neumann & Römheld 1999). Both chickpea and white lupin are in the family of the *Fabaceae*, same as *Medicago truncatula*. Another study comparing the exudation of low molecular weight organic acids (LMWOA) in *Solanum nigrum* and *Solanum lycorpersicum* showed that *S. lycopersicum* exudes in general less LMWOAs than *S. nigrum* (Bao et al. 2011). This combined information supports the theory that *Medigaco* is more efficient in dissolving soil P through the exudation of organic acids. The second and third repetition of my experiment show that the roots and the fungus need to be in contact with or the roots have to be near the apatite, to make a difference on phosphate uptake from apatite, since the plants in the second repetition showed less phosphate in root and shoot material in the apatite plants than in the phosphate fertilized plants. Although the fungus is able to take up phosphate from apatite in this system, as was determined in the One Arm Experiment, it has also been established that it is a slow process, which might take longer than the six weeks available to the plants here. Therefore, it is not surprising that the plants had less phosphate in root and shoot material. It was surprising, though, that they did not have less phosphate in the first repetition, which is a strong indication for an influence of the plant. Furthermore, the plants did not look unhealthy, though they were phosphate-limited. It seems that *Medicago* plants are more tolerant against phosphate deprivation. The only indication towards less healthy plants is the overall nutritional status of the plants in the second repetition. The AA plants had less nutrients than the PP plants, or in some cases also the AP plants. This trend could be seen for several micronutrients. It is possible that the lack of phosphate is responsible for this overall lack of nutrients, since it is the only correlation which can be seen.

8.2 Differences Between Mycorrhizal Measurement Techniques

The mycorrhization analyzed with Alexa staining and microscopy techniques was relatively high in all plants and repetitions and was not significantly influenced by the treatment of the plants. This provides a good basis for the experiment, as changes in gene expression are not influenced by the degree of overall mycorrhization, but by the other controlled factors in the experiment. The expression of the fungal marker genes *GiTefa* and *GiRNS* was also measured to evaluate the mycorrhiaztion of the roots. It is conspicuous that the degree of mycorrhization measured with both methods is not the same. The differences measured with the microscopy techniques are not significant throughout all three repetitions. For the fungal marker gene measurements, there are significant differences for all three repetitions, though just between some treatments. The differences showing in the gene expression are also not reflected in tendencies in the microscopic measurement of the mycorrhization. The slight differences showing with microscopy techniques appear in different treatments than for the gene expression. Though both techniques are used in the literature to evaluate the mycorrhization of *Medicago* plants, discrepancies between them have been determined before (Pivato et al. 2007; Gamper et al. 2008; Shi et al. 2012). To approach this problem, a detailed experiment on the comparability of qPCR with visual quantification techniques was conducted (Gamper et al. 2008). The study worked with primers from 18S rRNA from the nucleus and found a stable and reproducible correlation between the gene expression and the number of spores used for quantification. However, for extra- or intraradical mycelium, no correlation between the qPCR measurement of the markers and the hyphal length or abundance as well as arbuscular abundance in the roots could be found. As an explanation, an uneven fungal distribution within the roots as well as an uneven distribution of nuclei in the hyphae was suggested. Spores seem to contribute considerably more to the qPCR measurement of fungal structures than hyphae or arbuscules. In ecological research with ectomycorrhizal fungi, this specific problem has been discussed before (Avis et al. 2006). Other studies were able to produce a high correlation between the techniques using *Medicago truncatula* samples colonized with *R. irregularis* (Alkan et al. 2004; Isayenkov et al. 2004). However, Alkan et al. (2004) used DNA samples instead of RNA samples, whereas Gamper et al. (2008) tried both DNA as well as RNA samples. They also stated that the correlation differed for the arbuscules, vesicles and hyphal structures with the best outcome for vesicular abundance, and they included barely colonized roots, which strengthened the correlation. Gamper et al. (2008) also suggested that vesicular structures are more likely to correlate with the qPCR measurement, since they are more alike to spores than arbuscules or hyphae. However, my experiment cannot confirm this theory, since there was also no correlation for vesicular abundance and fungal marker expression. The study by Isayenkov et al. (2004) is more interesting, since they worked with DNA and RNA respectively. They also found a strong correlation between fungal marker

gene expression and mycorrhization of the plant determined via microscopy, especially for the RNA approach. The experiment, however, was just conducted for 58 days. On the last day, the correlation was weakened with still high values for overall mycorrhization, but a decrease in fungal marker expression, which was explained with statistical variations. It might be possible that the observed correlation is restricted to the early stages of mycorrhizal colonization and is not as distinct in older plants like mine. Though Gamper et al. (2008) also used relatively young plants, they worked with *Allium porrum* and *G. mossea*, and the factors influencing this correlation could be species-dependent. In conclusion and as suggested by Gamper et al. (2008), the differences in measuring fungal abundance with the two techniques used by us are most likely caused by targeting different biological units. Therefore, it might always be useful to evaluate the mycorrhization with both techniques to broaden the overall picture.

8.3 Influence of Mycorrhization and Phosphate Availability on *MtPT4* Expression

The *MtPT4* expression seems to reflect the fungal marker gene expression quite well. For each repetition, the expression patterns of *MtPT4* and the fungal marker genes correlate strongly with one another. The fold change in expression between the treatments is almost always nearly the same for both genes. There are still slight variations, especially in the significance of differences between the treatments. In the second repetition, for example, the expression between the A side of the AP plants and both sides of the AA plants is significantly different for the fungal marker *GiRNS*, whereas there is no significance for the same difference for *MtPT4*. Similar slight discrepancies in the statistics can be seen for the first and third repetition, though there is always a correlation between the expression of the genes. Even the standard deviations for both genes are quite similar.

This correlation could be explained with two theories. Either, one of the factors is directly influencing the expression of the other gene, for example the mycorrhization is influencing *MtPT4* expression. Or the transcript levels of the genes are both influenced by the same factors, which leads to similar expression patterns. Furthermore, a mixture of both theories seems possible. For further discussion, it is necessary to keep in mind that the general mycorrhization, as determined with microscopy, does not necessarily correlate with the fungal marker expression, as discussed before (Isayenkov et al. 2004; Gamper et al. 2008).

In their study, Isayenkov et al. (2004) also investigated the expression pattern of *MtPT4,* and found a correlation between it and both mycorrhization and fungal marker gene expression in the first 51 days of their experiment, but a strong decrease of *MtPT4* expression at day 58, though the roots were highly colonized. The fungal marker abundance also weakened after 58 days, though not as

strong as for *MtPT4*. Since the plants in my experiment were harvested 84 days after inoculation, it is possible that the discrepancies between *MtPT4* expression and mycorrhizal status of the plants only occur in later stages of the symbiosis. The same can apply for the fungal marker genes.

Isayenkov et al. (2004) claimed that the difference between *MtPT4* and fungal marker gene expression after 58 days was evidence that the qPCR measurement of the fungal marker did not reflect the symbiotic activity, which should be more related to the transport of phosphate and therefore the *MtPT4* expression. In their experiment, *MtPT*4 expression correlated strongly with the initial amount of inoculum as well as with the arbuscular abundance in the roots, which was also lower at day 58 compared to the previous harvestings. They also point out that *MtPT4* expression could be used as a marker for the symbiotic activity, especially under P deficiency, because of its link to the phosphate transport (Harrison et al. 2002). It has been shown that *MtPT4* is essential for a stable symbiosis (Javot, Pumplin, et al. 2007), which is why it is commonly regarded as a marker for symbiotic activity. A general correlation of *MtPT4* expression and root colonization as well as arbuscular abundance has also been found by Feddermann et al. (2008). However, they also found an additional correlation between *MtPT4* expression and the shoot P content, though not the root P content. The correlation of *MtPT4* expression with the arbuscular abundance seems logical, since the gene is only strongly expressed in arbuscule-containing cells (Harrison et al. 2002). This could be confirmed by a study applying laser microdissection techniques and using *MtPT4* as a marker for arbuscules. They were not able to find *MtPT4* transcripts in colonized cells without arbuscules (Hogekamp et al. 2011; Hogekamp & Küster 2013). However, later research using laser microdissection techniques shows that the transcript can also be detected in mycorrhized cells without arbuscules, though in smaller amounts (Gaude et al. 2012). In my experiment, both *MtPT4* expression and fungal marker gene expression, did not correlate with the arbuscular abundance measured with microscopic counting techniques. It is possible that the counting technique includes arbuscules which are already recessing and which are therefore not symbiotically active, which could lead to a bias in the analysis. Furthermore, it is also possible that the expression of *MtPT4*, though restricted to arbuscule containing cells, can differ in these cells due to several biotic or abiotic factors, as for example the fungal species, the phosphate transport or the P supply (Feddermann et al. 2008; Breuillin et al. 2010; Fiorilli et al. 2013; Fellbaum et al. 2014). Fellbaum et al. (2014) also described differing *MtPT4* transcript levels for shaded and non-shaded plants, while the arbuscular abundance in the roots remained relatively constant. It is therefore likely that the *MtPT4* expression in my experiment was also influenced by another factor than the arbuscular abundance, though a general influence cannot be excluded.

The second possible influencing factor, which was also the controlled factor in my experiment, is the phosphate availability and content. My hypothesis was that phosphate availability in the soil will

influence *MtPT4* expression. As mentioned above, other studies have found a connection between phosphate and the phosphate transporter expression (Feddermann et al. 2008; Fellbaum et al. 2014). Fellbaum et al. (2014) found a correlation of *MtPT4* transcripts and the P transport in the mycorrhized roots, as well as the P tissue concentration in a study with shaded and non-shaded *M. truncatula* plants. The shaded plants received less phosphate from the mycorrhizal fungi and showed a proportional decrease in *MtPT4* transcripts. However, the P supply of these plants was not otherwise limited (Fellbaum et al. 2014). Another study observed an increase in *MtPT4* expression coupled with a higher P transport to the mycorrhizal roots. An external supply of 320 µM Pi compared to 32 µM Pi led to an upregulation of *MtPT4* paired with a higher P content in the root material (Fiorilli et al. 2013). Isayenkov et al. (2004) even suggested that *MtPT4* expression can be used to determine the correlation between root colonization, external P supply and P transport by AM fungi. Since MtPT4 is the main phosphate transporter in the mycorrhizal symbiosis, this assumption seems plausible, though my experiments show that it is not necessarily true. In the tomato split root experiment, both the fungal marker and the *LePT4* gene reacted to a lack of phosphate with a change in expression, though clearly more distinct for the phosphate transporter. However, this correlation could not be seen in the repetition with *M. truncatula*. In all three repetitions, a correlation between phosphate supply and/or phosphate transport cannot be observed. These results refutes my hypothesis for *M. truncatula*. Though there are differences in *MtPT4* expression in my study between the treatments, it is not clear what caused these differences, in spite of the general mycorrhization as determined via the fungal marker expression. In the first repetition, the only significant differences were between the NP plants and the other treatments, with a higher expression in both sides of the NP plants. There was no significant difference in the phosphate status of these plants compared to the other treatments, which rules out the phosphate availability as a likely explanation. Furthermore, there was no difference between the two sides of the treatments in gene expression, although the phosphate availability was a controlled factor in the experiment and it differed severely. This underlines that the phosphate status of the plant seems not to influence both fungal marker and *MtPT4* expression, as it has in the tomato experiment. The expression differences seen in the experiment here cannot be explained by the controlled factors of the experimental set-up. Aside from the phosphate fertilization, the plants were treated in the exact same way and the randomized set-up with frequent changes in position in the phytochamber should exclude factors such as light or humidity as an influence. It is still a possibility, though, that an unknown, uncontrolled factor is responsible for the expression changes in the NP plants.

For the third repetition, the P side of the PN plants had the highest *MtPT4* expression whereas the PP plants showed the lowest expression, although both treatments received the same amount of phosphate. Furthermore, a correlation with the plant P status determined with the ICP measurement

could not be observed. The plant P status as well as the P availability in the soil therefore seems again not to be linked to either *MtPT4* expression or the expression of the fungal marker gene in *M. truncatula* in this experiment.

In the second repetition, there was a tendency towards a higher expression of *MtPT4* and the fungal marker gene on the A side of the AP plants. The other treatments did not differ much in their expression levels. Again, the P content did not correlate with the gene expression, as P status of the plants was highest in the PP plants and lowest in the AA plants. The arbuscular abundance, however, was by trend higher in the AA plants compared to the PP plants. In this repetition, the two discussed factors, arbuscular abundance and P content of the plant, are pronounced contrary to each other. This suggests the possibility that the *MtPT4* expression is influenced by both factors simultaneously. As discussed above, previous studies suggest both correlations (Feddermann et al. 2008; Isayenkov et al. 2004; Fellbaum et al. 2014; Hogekamp & Küster 2013; Hogekamp et al. 2011; Fiorilli et al. 2013). Therefore, the nearly constant transcript levels of *MtPT4* in this repetition could be an outcome of the two rivaling factors, both influencing *MtPT4* expression at once. The higher arbuscular abundance in the AA plants would lead to a rise in *MtPT4* transcript levels, whereas the lower phosphate status of the root would be coupled to a downregulation of the gene. A mixture of these, and maybe even more factors, could lead to *MtPT4* expression values, which show no clear correlation to one of the factors on its own, as seen throughout the whole experiment. The unclear correlations in the other two repetitions point towards another influencing factor, which is currently unknown to us and which was evidently not controlled in my experiment, since no other correlations could be observed. Furthermore, the distinct expression pattern in every repetition could not be reproduced. This solidifies the theory that the expression of *MtPT4* and the fungal marker respectively is influenced by factors not under control in this experiment, and not fully known to us. To unravel the other influencing components, it would help to understand why the expression of the fungal marker gene and the overall mycorrhization determined by microscopy are different from one another. A further understanding of the fungal marker gene expression and its influencing factors might also lead to a further insight in *MtPT4* expression.

In addition, my experiment shows that the PT4 transporters, though genetically very similar, can differ greatly in their expression pattern between different plant species. In the same experimental set-up using tomato plants, *LePT4* was strongly down-regulated in response to P starvation. A correlation to the fungal marker expression was also evident, but the fold-changes in the fungal marker expression were not as distinct in response to changes in P availability as for *LePT4* and could therefore not fully explain the different transcript levels between the treatments. I argued that, for tomato, *LePT4* is not an accurate marker for the mycorrhization of the plant, since the P status of the soil and the plant also have a great influence on its expression. It can therefore just be used as a

mycorrhizal marker in tomato plants if these factors are fully controlled. *MtPT4* seems to be more closely linked to the quantity of mycorrhization, as measured with fungal marker genes, than *LePT4* in tomato, since there was a very strong correlation between fungal marker gene expression and *MtPT4* throughout the whole experiment. Therefore, it should be carefully checked for each new species, how closely the *PT4* gene is linked to the expression of fungal marker genes, before it can be used as a common marker. The examples given with my experiments show that the correlation between the gene expressions is species dependent and can differ considerably. However, the *PT4* gene is still a valuable marker to determine if a mycorrhization is present, since there is no gene expression detectable in non-mycorrhized plants.

8.4 Influence of Phosphate Availability on Fungal Transporter Gene Expression

The expression patterns of the fungal genes *GiPT* and *GiMST2* were also determined. In the tomato experiment, the expression patterns of these genes were mostly very similar. They were both down-regulated in response to phosphate starvation and had an increased expression if the phosphate status of the plant and the soil were relatively low. For *GiPT*, this outcome supports the theory that the transporter is part of a competition for phosphate between the plant and the fungus at the periarbuscular membrane under P-deficient circumstances (Benedetto et al. 2005; Balestrini et al. 2007; Fiorilli et al. 2013). In the *Medicago* split root experiment, there was no serious P-deficiency. The plants were able to gain P from apatite in a nearly similar amount than from the P-fertilizer, at least in the first repetition. For the second and third repetition, the AA plants had less P in the roots and shoots than the PP plants, but all the plants looked healthy and had similar weights, which makes a serious P-deficiency unlikely.

The expression of *GiPT* and *GiMST2* in the *Medicago* split root experiment correlates again with one another, as already seen in the tomato experiment. Though the statistical differences are not always the same, the general expression pattern is very similar. In the first repetition, the *GiPT* expression also correlates mildly with the phosphate content of the roots. Though not statistically significant, the P side of the AP plants contains more phosphate. The *GiPT* and *GiMST2* expression of these treatments is higher for the A side, hence there is the same negative correlation as in the tomato plants. This supports the above-mentioned theory of the phosphate competition at the periarbuscular membrane.

The data for the second repetition is not as clear, since there are no statistical differences in between the treatments for both fungal genes. The third repetition shows differences, but there is no correlation to the P status of the roots. However, both the second and the third repetition have been

done in a different experimental design than the first repetition or the tomato experiment. That might explain the differences observed between these repetitions. Furthermore, as said before, a severe P-deficiency could not be observed in the plants. It is also possible that all plants only suffered a minor P-deficiency, which did not affect them seriously. Most important, there were no big differences between the plants concerning their P status.

The detectable expression of *GiPT* shows again, as before in the tomato experiment, that *GiPT* is expressed in the intraradical mycelium, which is in accordance with data from the literature (Benedetto et al. 2005; Balestrini et al. 2007; Gómez-Ariza et al. 2009; Tisserant et al. 2012; Fiorilli et al. 2013; Harrison & van Buuren 1995). Contrary to other studies, where the fungal phosphate transporter was expressed at a constant level in the arbusculated cells of the plant (Benedetto et al. 2005), the expression of *GiPT* varied in my experiments, in tomato as well as in *M. truncatula*. Since all the plants were fertilized with a minimum amount of phosphate, it is possible that all of them suffered from a low P-deficiency. If *GiPT* is only expressed in recognizable amounts in the intraradical mycelium under P-deficient conditions, which lead to a competition for P, the *GiPT* expression in this experiment would suggest that all plants suffered a P deficiency, albeit a minor one, which did not affect their phenotype. In the third repetition, the PP plants and the P sides of the AP and PN plants showed a tendency towards a slightly or even significantly lower *GiPT* and *GiMST2* expression. This would further support the above mentioned theory, since these roots and fungi would suffer the least from P deficiency compared to the other treatments. Though this experiment does not further verify the competition theory, my findings are not in opposition to it, either. The outcome of all my experiments rather supports the theory than disprove it.

The expression of *GiMST2* has been reported in the literature to correlate with the *PT4* expression of the host plant, since both genes were downregulated in response to high phosphate fertilization (Helber et al. 2011). Another study reported an upregulation of *GiMST2* under low phosphate conditions compared to moderate phosphate fertilization, whereas the *PT4* gene was upregulated under the moderate conditions (Fiorilli et al. 2013). In the *Medicago* split root expriment, a correlation of *GiMST2* with *MtPT4* expression could not be observed, neither a positive nor a negative one. The transcript level of *GiMST2* seems to act independently from the *MtPT4* expression. Since both possibilities have been described in the literature, these findings do not oppose a common theory, but add to the existing knowledge about *GiMST2* regulation. My experiment further supports the theory that a general correlation between *GiMST2* and *MtPT4* is unlikely. Helber et al. (2011) suggested a link between *GiMST2* expression and the symbiotic phosphate delivery to the plant, measured via *MtPT4* expression. Though my findings cannot support this theory, since this correlation could not be confirmed, I also found no direct correlation between phosphate delivery and *MtPT4* expression in this experiment. Therefore, my data does not necessarily refute this theory,

either. *GiMST2* expression can still be connected to the phosphate delivery, although this might not be reflected by the *MtPT4* expression. As described for *GiPT*, there is a slight correlation between *GiMST2* and phosphate availability, with a lower expression of the fungal gene if more phosphate is available. This is in accordance with the findings from Fiorilli et al. (2013), stated above. In conclusion, the data derived in the *M. truncatula* experiment mostly support the line of discussion from the split root experiment with tomato. The correlation with the phosphate content is not as distinct as in the tomato experiment, but as discussed before, the *Medigaco* plants did not suffer as much under the P-deficiency as the tomato plants, which could definitely influence the fungal transporter expression. A species-relation of these symbiotic genes is also possible and, as mentioned above, not uncommon.

8.4 Fatty Acid Composition in the Roots of *Medicago truncatula* in Response to Different Phosphate Availabilities

To further support my data, a ^{13}C labeling of the third repetition was done three days prior to the harvesting. The labeling, in combination with an extraction of the fatty acids from the root material, was supposed to give insight in the C distribution from the plant to the fungus under the influence of different P sources. Furthermore, the analysis of both the neutral and phospholipid fatty acids can show if the plant derived C is invested in either growing structures or storage organs of the fungus.

The ^{13}C measured in the shoot material showed no difference between the five treatments. As expected, the P source had no influence on the uptake of gaseous ^{13}C and therefore on the general photosynthesis of the plant. The analysis of the root bulk ^{13}C also showed no statistical differences, but there was a tendency towards a higher $\delta^{13}C$ level in the plants supplied with phosphate. It seems possible that the C allocation towards the roots is slightly increased if more phosphate is available to the plant roots. This stands against the reports of an increased C allocation towards the roots in response to Pi starvation (Liu et al. 2005; Hermans et al. 2006; Hammond & White 2008; Lemoine et al. 2013). However, as explained above, my *Medicago* plants did not suffer under severe Pi starvation. Furthermore, an increase in ^{13}C enrichment of mycorrhized roots in response to P fertilization has also been observed by Olsson et al. (Olsson et al. 2002; Olsson et al. 2006). Though the preceding experiments show that the fertilization with apatite instead of phosphate provides the *Medicago* plants with enough phosphate to strive, the direct phosphate fertilization still leads to a slightly better phosphate status of the plant and is more effective for plant fertilization. Although the root phosphate status showed no significant differences either, a tendency towards a higher phosphate status in the P fertilized plants was also visible in the third repetition. A trend towards a

higher C allocation from the shoot to the mycorrhizal root, if more phosphate is available, therefore seems plausible.

To evaluate the mycorrhization of the roots with the help of lipid fatty acids, the fatty acid 16:1ω5c is commonly used as a marker for AM fungi (Müller et al. 1994; Larsen et al. 1998; Olsson 1999; Gavito & Olsson 2003). Its abundance as PLFA and NLFA gives insight in the amount of growing structures such as hyphae and arbuscules, where PLFAs are mainly incorporated in the membranes (Olsson & Johansen 2000; Aarle & Olsson 2003), or vesicles and spores where NLFAs are incorporated in storage lipids (Graham et al. 1995; Olsson & Johansen 2000; Aarle & Olsson 2003; Bago, Zipfel, et al. 2002). The measurement of both the fungal PLFA and NLFA can help determine the state of the mycorrhizal symbiosis, since the ratio between neutral lipid and phospholipid content can be used as an indicator of C allocation to energy storage in AM fungi (Olsson 1999). The abundance of NLFAs is typically higher than of PLFAs in AM fungi, since they store large amounts of their carbon as neutral lipids (Olsson & Johansen 2000). I can confirm this observation, since the total amount of the 16:1ω5c NLFA in the root samples was in general 100 times higher than the amount of the 16:1ω5c PLFA in my experiment.

8.4.1 Fungal PLFA Abundance in *Medicago truncatula* Roots in Response to Changes in Mycorrhzation and Phosphate Availability

The amount of the 16:1ω5c PLFA in my experiment did not change significantly between the treatments. The only difference was within the NP plants, with a higher amount of PLFA on the P side of the treatment. This is mirrored by a tendency towards a higher abundance of arbuscules on the P side of the NP plants, determined with Alexa staining and microscopy techniques. These findings support the usage of the 16:1ω5c PLFA as a marker for arbuscular abundance. A very close correlation between PLFA accumulation and arbuscular abundance has also been observed in a past study by Aarle & Olsson (2003). In my experiment, the NP plants certainly have the most drastic difference between their two sides, with the best and most effective phosphate source on one side and no phosphate available to the fungus on the other side. It seems plausible that the plant invests more into the fungus in a root region where P is delivered to the roots in relatively large amounts, than in regions where no trading takes place. The differences in P supply between the sides of the other treatments might be not as drastic as for the NP plants, and therefore no difference in PLFA abundance and arbuscule accumulation occurs. Overall, the relatively similar amount of PLFA in all treatments indicates that the P source has no major influence on the growing structures of the fungus inside the roots, as long as P is available.

The ratio of $\delta^{13}C$ within the fatty acid and the root material show a similar picture. There are nearly no differences within or between the treatments for the PLFA $\delta^{13}C$ ratio. This indicates strongly that the distribution of carbon from the plant to the fungus is not influenced by the source of P available to the fungus. Though the abundance of the fungal PLFA was higher on the P side of the NP plants, there was no reduction in ^{13}C in the PLFA on the N side of the NP plants compared to the general amount of ^{13}C in the roots. The carbon delivered to the fungus from the plant was not reduced due to the lack of available P. However, the fungus produced less PLFA and therefore growing structures on the side without P compared to the P-treated side. The decrease in fungal structures consequently seems to derive from the fungus, not from a decrease in carbon allocation by the plant.

8.4.2 Fungal NLFA Abundance in *Medicago truncatula* Roots in Response to Changes in Mycorrhization and Different Phosphate Availabilities

In previous studies, a correlation of 16:1ω5c NLFA accumulation in the roots and the microscopically estimated total root colonization has been observed (Olsson et al. 1997). However, NLFAs are typically found in storage organs such as vesicles and spores, which is why a correlation with these structures also seems likely. My experiment, though, shows only a low correlation between vesicle abundance, as determined with microscopy and 16:1ω5c NLFA accumulation. For example, the 16:1ω5c NLFA abundance is especially high on the P side of the AP plants, while the vesicular abundance is nearly the same for both sides. However, Aarle & Olsson (2003) also found that the abundance of vesicles and the 16:1ω5c NLFA was not as closely correlated as for the corresponding PLFA and the arbuscular accumulation. In their experiment, the NLFA abundance increased continuously throughout the 60 day period, whereas the vesicle abundance peaked after 32 days and decreased again. Furthermore, in the beginning of the experiment, vesicles could already be observed, although very little of the NLFA could be found. It seems that the formation of vesicles does not coincide with the accumulation of storage lipids. This observation was also made in another study with citrus and *G. intraradices*, where the lipid accumulation occurred after the vesicular colonization as well (Graham et al. 1995). It was postulated that vesicles are built before lipid accumulation occurs and are filled with storage lipids at a later point (Aarle & Olsson 2003). A second study by Graham et al. (1996), where NLFAs continued to accumulate after vesicle formation did not further increase, supports this theory. Therefore, a low correlation between vesicle abundance and NLFA accumulation is not totally unexpected. Furthermore, neutral lipids are not only produced to store energy in structures like vesicles, but also to transport carbon form the intra- to the extraradical mycelium (Bago, Zipfel, et al. 2002). Hence, not all NLFAs extracted from the roots are

necessarily obtained from vesicles and spores, which will further negatively influence a possible correlation.

In general, the abundance of the 16:1ω5c NLFA shows more difference within and between the treatments than the PLFA. The NLFA to PLFA ratio, which can also be used as a marker of C allocation to fungal storage structures (Olsson 1999), shows a very similar pattern to the 16:1ω5c NLFA abundance, which is why I concentrated on the amount of NLFA. There is a significant difference between the A and P side of the AP plants, with more NLFA on the P side. The AN plants also show a tendency towards a lower amount of NLFA on the A side. The AA and PP plants, however, show no difference between the sides or to each other. It seems that the NLFA abundance can be influenced in different root regions, and both P availability and P source are a possible factor, but the experimental results cannot be interpreted in a definite way. Other studies found a decrease of 16:1ω5c NLFA in the roots in response to P fertilization. However, in both studies the P fertilization was supposed to lead to a less beneficial situation in the symbiosis by adding large amounts of P (90 mg/kg soil) (Olsson et al. 2006; Olsson et al. 2010). My plants received small amounts of P instead, which did not have a negative effect on the mycorrhizal symbiosis, especially because only the fungus had access to the P source. I observed a higher NLFA abundance on the P side of the AP plants, but also on the N side of the AN plants, which shows that the amount of P available to the fungus cannot be the only influencing factor, at least not in a scale as narrow as in my experiment. If only P availability would be the pivotal factor, the A side of the AN plants would have a higher amount of NLFA instead of the N side. The lower NLFA abundance on both A sides of the AN and AP plants could lead to the impression that the source of P, in this case apatite, is influencing the amount of 16:1ω5c. However, the similar NLFA abundance in the AA and PP plants also disproves this theory. Only differences in P source within a root system lead to different NLFA distributions, while the amount of NLFA is similar in plants fertilized with only one P source. Further experiments may be necessary to evaluate the abundance of 16:1ω5c NLFA in relation to P source and availability in different soil patches in the same root system. The theory that plant C allocation can react specifically to P-enriched patches in the soil was also postulated by Olsson et al. (2006). The study reckoned that mycelial proliferation in P-enriched patches might depend on plant C allocation specifically for this patch. Therefore, C supply and P supply would be linked indirectly through the plant. My data would support this theory.

The differences in the δ^{13}C NLFA/ δ^{13}C root ratio are also not statistically significant. However, where the PLFA ratios were quite similar, the NLFA ratios show tendencies towards differences in abundance between the treatments. It seems that the C investment into NLFAs is more variable then into PLFAs within a functional symbiosis. The AA plants have a lower δ^{13}C NLFA to δ^{13}C root ratio

compared to the other treatments, and there are also differences within the AP and NP plants. Again, the influence is unclear, since in the NP plants, the phosphate side has a higher $\delta^{13}C$ abundance in the NLFA compared with the $\delta^{13}C$ in the roots, whereas for the AP plants, the P side shows less $\delta^{13}C$ abundance in the NLFA. The P source or P availability might not be the crucial factors in the incorporation of plant derived C into the storage structures. Since the $\delta^{13}C$ ratios did not differ for the PLFA, it seems possible that the differences within the $\delta^{13}C$ NLFA accumulation is not based on a difference in carbon delivery from the plant, but on the distribution of the plant derived carbon inside the fungal structures. The total amount of NLFA was highest in the P side of the AP plants, whereas this side shows the lowest $\delta^{13}C$ NLFA to root ratio. This suggests a low production of storage lipids in the three days after the labeling in this root section, however the general amount of NLFA in these plants show that there was no shortage of storage lipids in these roots. Vesicles last longer than fast growing structures such as arbuscules, which are believed to have a turn-over rate from one to two weeks (Alexander et al. 1989). Therefore, a constant investment of plant derived carbon into growing structures is needed, whereas vesicles can be build and filled with lipids as needed. The similar abundance of PLFA and the constant $\delta^{13}C$ PLFA/$\delta^{13}C$ roots ratio in contrast to the more variable data for the NLFA in my experiment is in line with this explanation. In root parts which already have a high accumulation of NLFAs, like the P side of the AP plants, an additional investment of C into NLFAs is not needed, hence the low $\delta^{13}C$ NLFA/$\delta^{13}C$ root ratio. In contrast, root sections with a low NLFAs abundance invested more into newly build NLFAs. The $\delta^{13}C$ data therefore further supports the theory from Aarle & Olsson (2003), discussed above, suggesting that vesicles are built before lipid accumulation takes place, and they are filled with storage lipids at a later time. Where the PLFA abundance and the investment of plant derived carbon is steady and very similar in all root parts, independent of the phosphate treatment, the abundance as well as the distribution of plant derived carbon in NLFAs seems to be more variable and prone to influences. However, the main influencing factor in NLFA production cannot be identified in this experiment.

9. Relation of the Three Experiments and Connection of Their Results

Overall, my first experiment shows that the expression of the tomato phosphate transporters *LePT4* and *LePT3*, although linked to a certain degree to the mycorrhization, is also greatly influenced by the phosphate status of the surrounding soil, especially if huge differences occur in phosphate availability between plants or root patches. The second part of the tomato split root experiment showed that *LePT4* expression can also differ from the degree of mycorrhization. This was further confirmed by the One Arm Experiment, where *LePT4* and the fungal marker gene expression did not correlate for most of the treatments. Therefore, other factors must have an influence on *LePT4* expression. The One Arm Experiment showed that the phosphate source, as suggested in my initial hypothesis, is not an influencing factor. This was not clear after the split root experiment, since the apatite fertilized plants suffered from severe Pi starvation. The One Arm Experiment lasted long enough for the fungus to gain P from the apatite and no differences in *LePT4* expression between the phosphate sources could be observed. The phosphate availability and especially phosphate starvation, though, is clearly influencing *LePT4* expression, which was demonstrated in the tomato split root experiment. The comparison of these two experiments suggest that small changes in P availability have no influence on *LePT4* expression, whereas large differences will make an impact. *LePT3* expression reacts similar, but the changes are not as distinct as for *LePT4*. Therefore, the phosphate status of the soil and the plant has to be taken into account, if *LePT4* should be used as a marker for mycorrhization, as suggested by Javot et al. (2007). Relying solely on *LePT4* expression to determine the degree of mycorrhization can be misleading. Furthermore, *LePT4* and *LePT3* should also not be used as markers for the P flux in the mycorrhizal system or the correlation between colonization, external P supply and P transport by the fungus, which was proposed by Isayenkov et al. (2004), since small changes in P availability cannot be detected.

In the split root experiment with *M. truncatula*, a correlation of *MtPT4* expression with the degree of mycorrhization could be seen in all three biological replicates, but a correlation with the phosphate status, as in tomato, could not be observed. This rebuts the hypothesis that the phosphate status has an influence on *MtPT4* expression, as in tomato. However, the experiment supports the common usage of *MtPT4* as a marker for mycorrhizal colonization, at least when compared to the expression of other fungal marker genes. The experiment also confirmed the findings of other studies, showing that the mycorrhizal measurement via fungal marker expression and staining coupled with microscopy did not necessarily lead to the same results (Pivato et al. 2007; Gamper et al. 2008; Shi et al. 2012). This leads to the assumption that the two techniques are targeting different biological units, which do not necessarily correlate with each other. *MtPT4* and the common fungal marker genes, however, are most likely influenced by the same factors.

The comparison of the two split root experiments showed that the regulation of the *PT4* gene is most likely species dependent. Thus, it is necessary to thoroughly test its correlation to root colonization and fungal marker gene expression in each new species, before considering it as a mycorrhizal marker. However, *PT4* expression is still a valuable tool to check for the general presence of a mycorrhizal symbiosis, since nearly no gene expression is detectable in non-mycorrhized plants.

The expression pattern of the two sugar transporters *SlSUT1* and *SlSUT2* is supposedly not influenced by either the phosphate status of the soil nor of the roots. Both the tomato split root experiment and the One Arm Experiment confirmed this, since no correlation to the phosphate sources or the phosphate availability could be observed. Furthermore, their expression might also not be as heavily influenced by the mycorrhization as proposed in the literature (Boldt et al. 2011). In the tomato split root experiment, *SlSUT2* showed no changes in expression between the mycorrhized and non-mycorrhized plants, whereas *SlSUT1* was even downregulated in the mycorrhized compared to the non-mycorrhized plants. However, both transporters can still be of importance for the symbiosis, since it is possible that the regulation of these sugar transporters is post-transcriptional or even post-translational and can therefore not be tracked by expression analysis (Bitterlich et al. 2014). Furthermore, their changes in expression during the mycorrhizal symbiosis can be species related. Another explanation could be that the carbon trading of the plant is instead regulated by different sugar transporters not yet known to us, by plant invertases or even other unknown proteins. In further experiments, it would be useful to analyze the proteome in addition to the RNA expression of the genes, to determine changes in protein abundance.

The *LePT4* and *GiMST2* expression, though proposed to be linked (Helber et al. 2011), must be controlled by different factors, at least under Pi starvation. In both tomato split root experiments, no positive correlation between the expressions of those genes could be observed. This was confirmed by the experiment with *M. truncatula*, where no correlation between *GiMST2* and *MtPT4* expression could be observed, either. Instead, *GiMST2* expression seems to be negatively influenced by the phosphate status of the plant or the soil, since an upregulation in response to Pi starvation was found in both experiments. Therefore, *GiMST2* expression could still be linked to the phosphate delivery towards the plant, though this is not reflected by the *PT4* expression. It is also possible that another fungal sugar transporter is involved in the mycorrhizal trading, which is not yet discovered.

In both split root experiments, an upregulation of *GiPT* in response to phosphate starvation could be observed, though most pronounced in the strongly P-deficient tomato plants of the split root experiment. The experiment therefore proves that *GiPT* transcripts can be detected in the intraradical mycelium and provides further evidence for a competition for phosphate at the plant fungal interface, as proposed by Benedetto et al. (2005).

Apatite can be used as a phosphate source for mycorrhized plants, but it can only serve as a long-term fertilization. The mining of apatite by the plant or the fungus is a relatively slow process. Differences in the ability of the plants to mine phosphate from the mineral could be observed. When in direct contact with apatite, the *Fabaceae M. truncatula* did not suffer at all from P starvation, if fertilized with apatite instead of phosphate solution, whereas the *Solanaceae Solanum lycopersicum* showed evident signs of starvation. However, both the One Arm Experiment, as well as the second design of the split root experiment with *M. truncatula*, demonstrated that apatite can be used as a phosphate source by the AM fungus *R. irregularis* in a sufficient manner. The same has been shown for phytate in the One Arm Experiment. For both of these phosphate sources, the C investment of the plant towards the fungus to gain the same amount of P was higher than for the easily accessible phosphate solution. Moreover, different kinetics could be observed between the phosphate sources, with more time necessary to achieve the same amount of P from apatite than from phytate or the phosphate solution. This indicates different uptake mechanisms for the mineral and the organic source. The weathering of apatite is most likely achieved by an active secretion of organic acids from the mycorrhizal fungus, whereas for the uptake of phosphate from phytate, a secretion of enzymes is most plausible. These different mechanisms would explain the increased carbon investment of the plant towards the fungus in the One Arm Experiment as well as the different kinetics in P uptake for the two P sources.

The ^{13}C labeling and PLFA and NLFA analysis of the *M. truncatula* plants from the split root experiment further showed that the investment in growing structures, such as arbuscules, is relatively constant, independent of the P source and availability. Differences could mainly be observed in NLFA abundance and therefore storage structures and lipids, especially if differences in phosphate availability within the root system occurred. The experiment also further supports the theory that vesicles are built first and filled with storage lipids at a later time point (Aarle & Olsson 2003).

All three experiments complement each other and the results helped to understand and discuss the results from the other experiments, respectively. Still, further experiments as described in the sections before, are necessary to fully comprehend the complex regulation systems of the transporter genes involved in C for P trading in the symbiosis as well as the C investment in response to different phosphate availabilities. Detailed knowledge of these processes will help to solve future problems in phosphate fertilization, since it can become a seriously limited resource.

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Appendix

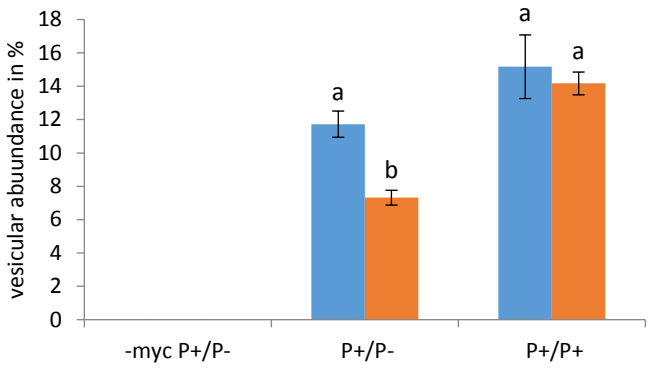

Figure X.1: Vesicular abundance of the plant roots from the first split root experiment with *S. lycopersicum*. Plants were 12 weeks old with 6 weeks in the split root system. Vesicular abundance was obtained using ink staining and microscopy techniques. -myc: nonmycorrhized plants; P+/P-: plants fertilized with 0.3 mM Pi on the P+ side and no phosphate on the P- side; P+/P+: plants fertilized with 0.3 mM Pi on both sides. Each pair of columns represents a split root system with one column per compartment. Data are given as mean values +/- SE (n=6; P+/P+ n=10; P+/P-, n=25). Different letters indicate significant differences ($p<0.05$).

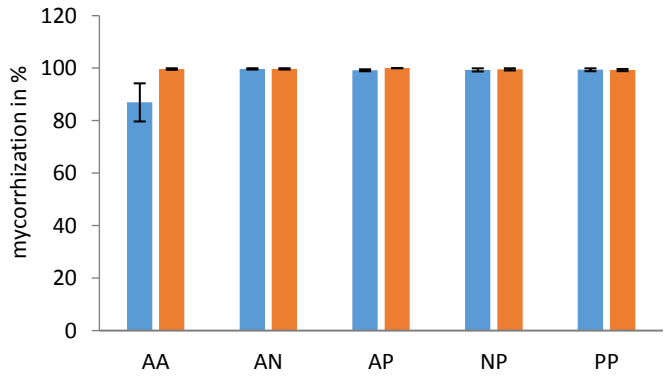

Figure X.2: Degree of mycorrhization in the roots of the first split root experiment with *M. truncatula*. Mycorrhizal status was determine via Alexa stainng and microscopy. Each pair of columns represents a split root system with one column per compartment. Blue bars: side with the first letter of the combination; orange bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

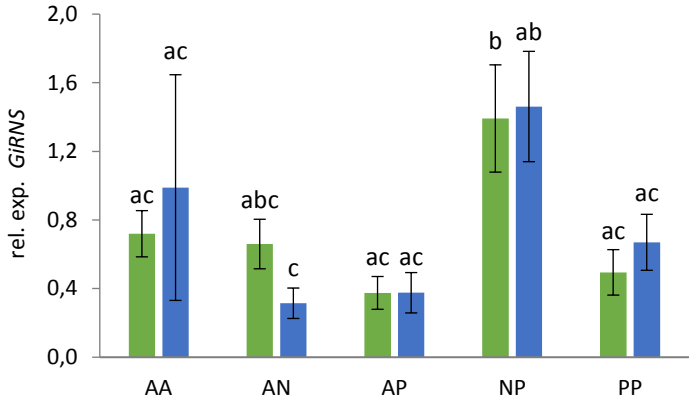

Figure X.3: Relative expression of *GiRNS* in the first split root experiment with *M. truncatula*. RNA expression was measured via RT-qPCR. *MtTef*α was used as a normalizer. Each pair of columns represents a split root system with one column per compartment. Green bars: side with the first letter of the combination; blue bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

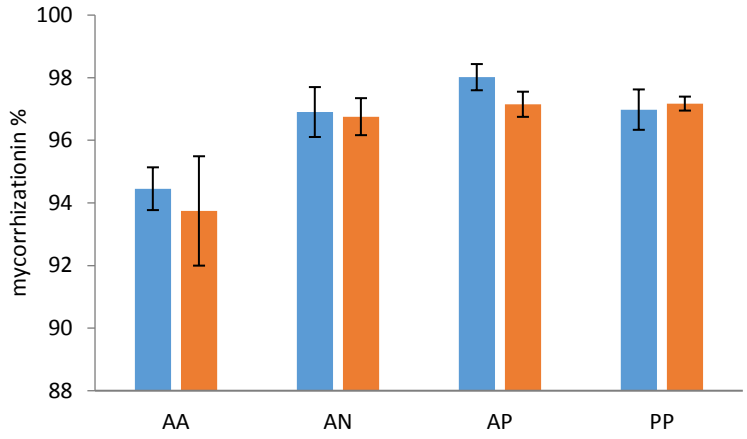

Figure X.4: Degree of mycorrhization in the roots of the second split root experiment with *M. truncatula*. Mycorrhizal status was determine via Alexa stainng and microscopy. Each pair of columns represents a split root system with one column per compartment. Blue bars: side with the first letter of the combination; orange bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

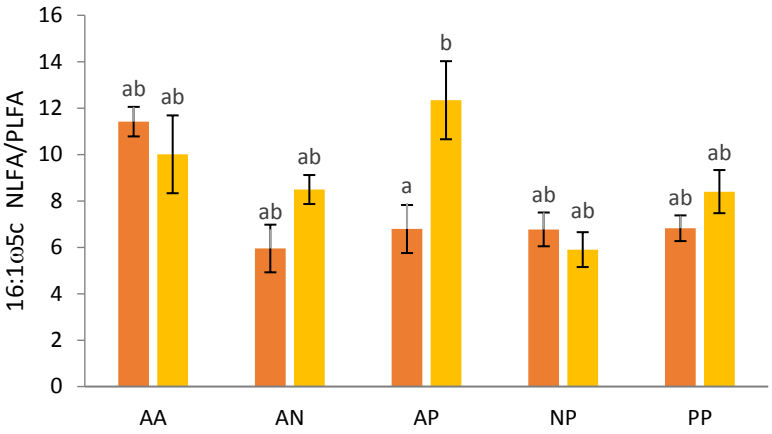

Figure X.5: 16:1ω5c NLFA/ PLFA ratio of the content from the roots in the third split root experiment with *M. truncatula*. PLFA and NLFAs were extracted from 100 mg ground and frozen root material. Each pair of columns represents a split root system with one column per compartment. Orange bars: side with the first letter of the combination; yellow bars: side with the second letter of the combination below. Data are given as mean values +/- SE (n=5). Different letters indicate significant differences ($p<0.05$).

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Conference Contributions

Oral Presentation

Jahrestagung der Deutschen Bodenkundlichen Gesellschaft 2015, München (09/2015)

„Regulierungs- und Steuerprozesse des Kohlenstoff- und Nährstoffaustausches an der Schnittstelle Mykorrhiza“

Poster Presentation

3rd Doctoral Researchers Conference 2016, Bad Salzdetfurth (09/2016)

“Modulation and controlling processes at the mycorrhizal interface in context of plant nutrient and carbon balance”

The 12th Solanaceae Conference, Bordeaux (10/2015)

“Modulation and controlling processes at the mycorrhizal interface in context of plant nutrient and carbon balance”

2nd Doctoral Researchers Conference 2015, Goslar (10/2015)

“Modulation and controlling processes at the mycorrhizal interface in context of plant nutrient and carbon balance”

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