Dynamics of carbon and phosphorus flows in arbuscular mycorrhizal symbiosis

mgr. Tereza Konvalinková

Doctoral thesis

Supervisor: Mgr. Jan Jansa, Ph.D.

Prague, 2017
Prohlášení:
Prohlašuji, že jsem předloženou disertační práci zpracovala samostatně a všechny použité zdroje jsem řádně ocitovala. Tato práce ani její podstatná část nebyla předložena k získání jiného nebo stejného akademického titulu.

Statement:
This dissertation describes my original work except where acknowledgement is made in the text. It is not substantially the same as any work that has been, or is being submitted to any other university for any degree, diploma or any other qualification.

V Praze, 4. 7. 2017

Podpis/Signature
# Table of contents

1 Abstrakt (in czech) .............................................................................................................. 6
2 Abstract ............................................................................................................................... 7
3 Abbreviations ....................................................................................................................... 8
4 Introduction .......................................................................................................................... 8
4.1 The general principles of mycorrhiza .............................................................................. 8
4.2 Arbuscular mycorrhiza and arbuscular mycorrhizal fungi ............................................ 8
4.3 Nutrient uptake by arbuscular mycorrhiza ..................................................................... 10
4.4 Carbon flow to arbuscular mycorrhizal fungi ............................................................... 12
4.5 Outcome of arbuscular mycorrhiza – an interplay between partners and environment .13
4.6 Partner discrimination and common mycorrhizal networks .................................................. 15
5 Aims and hypothesis .............................................................................................................. 17
6 Summary of published and unpublished results ................................................................. 19
6.1 Publication 1 – Konvalinková *et al.* (2015), published .............................................. 19
6.1.1 Statement of contribution ....................................................................................... 20
6.2 Publication 2 – Konvalinková and Jansa (2016), published ........................................... 20
6.2.1 Statement of contribution ....................................................................................... 21
6.3 Publication 3 – Řezáčová *et al.* (2017a), in press ......................................................... 21
6.3.1 Statement of contribution ....................................................................................... 22
6.4 Publication 4 – Konvalinková *et al*., submitted .......................................................... 22
6.4.1 Statement of contribution ....................................................................................... 23
6.5 Abstract of an unpublished experiment ........................................................................... 24
6.5.1 Statement of contribution ....................................................................................... 25
7 Discussion .............................................................................................................................. 25
7.1 Light availability as a basis of symbiotic functioning of arbuscular mycorrhiza .......... 25
7.1.1 Plants adapt themselves to C sink of AMF ................................................................ 25
7.1.2 How fast are changes in symbiotic flows after sudden light-shortage? ...................... 26
7.1.3 What can AMF do if their C income is lowered? ......................................................... 27
7.1.4 Importance of sufficient light availability for studies on plant symbiotic interactions ........................................................................................................ 29
7.2 Carbon cost of arbuscular mycorrhiza – what do we really know? ............................... 30
7.3 Arbuscular mycorrhiza under P-fertilization ................................................................. 31
8 Conclusions .......................................................................................................................... 34
9 Závěry (in czech) ................................................................................................................ 35
10 References .......................................................................................................................... 36
11 Acknowledgement ............................................................................................................... 42
12 Appendix ............................................................................................................................. 43
12.1 Publication 1 .................................................................................................................... 43
12.2 Supplementary material for Publication 1 ...................................................................... 43
12.3 Additional figures for Publication 1 ................................................................................ 43
12.4 Publication 2 .................................................................................................................... 43
12.5 Supplementary material for Publication 2 ...................................................................... 43
12.6 Publication 3 .................................................................................................................... 43
12.7 Publication 4 .................................................................................................................... 43
12.8 Supplementary material for Publication 4 ...................................................................... 43
12.9 Abstract of an unpublished experiment ......................................................................... 43
12.10 Statements of contribution ............................................................................................ 43
1 Abstrakt (in czech)

Arbuskulárně mykorrhizní (AM) houby jsou značně rozšíření a vysoce specializovány
kořenoví symbionti, již získávají veškerý svůj uhlík (C) z hostitelských rostlin, jímž na
oplátku poskytují minerální živiny (zejména fosfor, P). Tato práce je zaměřena na rozsah
a flexibilitu toků C a P v arbuskulární mykorrhizě vzhledem k podmínkám prostředí,
zvláště dostupnosti světla a P. Práce dále diskutuje názvky, že symbiotické toky jsou
aktivně regulovány oběma partnery. Poznámky jsou prezentovány ve formě souboru
vědeckých prací (dva původní články, jeden souborný článek a jedna
knihovní kapitola).

Jak přínosy mykorrhizy pro tolici (Medicago truncatula), tak její kolonizace AM houbou
(R. irregularis) klesala ve skleníkovém pokuse podél gradientu klesající intenzity světla.
Kupodivu, morfologická přizpůsobení tolice k dlouhodobému nedostatku světla byly dále
posíleny mykorrhizou, pravděpodobně kvůli požadavkům AM houby na C a díky lepší
výživě mykorrhizních rostlin. Na druhou stranu, náhlé šestidenní zastínění vedlo
to rychlému poklesu obsahu P v prýtech mykorrhizních rostlin, doprovázenému akumulací
P v kořenech. To napovídá, že AM houby pokračovaly v čerpání P z půdy, ale
neposkytovaly ho hostitelí, možná jako „trest“ za nízkou dodávku C. Experiment
s kompetujícími rostlinami (tolice a pórem, Allium porrum) sdílícími mykorrhizní síť s
ukázal, že mykorrhizní tok P může být rychle přesměrován z jednoho hostitele na
druhého v reakci na náhlu změnu světelných podmínek. Následný průzkum odborné
literatury odhalil propastný nedostatek znalostí o vlivu krátkodobých změn osvětlení
(jako například dešťových dnů) na fungování mykorrhizy.

Tři druhy rostlin (tolice, pór a jilek, Lolium perenne) byly inokulovány komplexním
společenstvem AM hub a vystaveny dvěma úrovním P v půdě. Tok C byl značen
stabilním izotopem $^{13}\text{C}$; alokace C do 16:1 mastné kyseliny zastupovala tok C do AM
hub. Hnojení P snížilo mykorrhizní přínosy i tok C do AM hub. Co je však důležité,
snížení toku C do hub bylo nezávislé na toku C do kořenů a sestávalo jak ze sníženého
množství AM hub, tak ze sníženého příjmu C na jednotku houby, což naznačuje aktivní
úlohu rostliny. Vedle toho byl podíl AM hub na celkovém rozpočtu C hostitelů (cena
mykorrhizy) porovnán s dřive publikovanými hodnotami; práce zdůrazňuje, že uhlíková
cena mykorrhizy je nejčastěji pod 10 %, ačkoliv vyšší hodnoty jsou časté (a někdy
nesprávně) uváděny sekundární literaturou.

Závěrem, toky P od AM hub reagují rychle na náhlé změny osvětlení, což otevírá možnost,
že AM houby disponují vyjednávací silou, třebaže jsou zcela závislé na svých hostitelích.
Na druhou stranu, pokles příjmu C AM hub v půdě hnojené P naznačuje, že hostitel
aktivně potlačuje tok C do nepotřebného symbionta. Práce vyzdvihuje důležitost
světelných podmínek pro fungování mykorrhizy a neuspokojivé znalosti o skutečné
uhlíkové ceně arbuskulární mykorrhizy.

Klíčová slova: arbuskulární mykorrhiza, náklady a přínosy, uhlík, hnojení fosforem,
dostupnost světla, sdílené mykorrhizní síť, značení stabilními izotopy
2 Abstract

Arbuscular mycorrhizal fungi (AMF) are widespread and highly specialized root symbionts, which gain all of their carbon (C) from the hosts, supplying plants with mineral nutrients (particularly with phosphorus, P) in return. This thesis focuses on the size and flexibility of C and P flows in arbuscular mycorrhiza in relation to environmental conditions, in particular to light and P availability. The indications that the symbiotic flows are regulated actively by both partners are discussed. The main findings are presented as a compilation of separate scientific works (two research articles, one review and one book section).

A glasshouse experiment has shown that both mycorrhizal benefits and mycorrhizal colonization of medic (Medicago truncatula) by an AMF species (R. irregularis) decline along the gradient of decreasing light intensity. Interestingly, morphological adaptation of medic to the long-term light deprivation was boosted by mycorrhiza, probably because of C demand of AMF and due to the improved nutrition of the mycorrhizal plants. On the other hand, sudden 6-day shading caused rapid decline of shoot P content of mycorrhizal plants, accompanied with the accumulation of P in the roots. This suggests that the AMF continued to take up P from the soil but did not supply it to the host, possibly “punishing” low C supply from host. An experiment with competing plants (medic and/or leek, Allium porrum) sharing common mycorrhizal networks showed that mycorrhizal P flows can be quickly redirected from one host to another in reaction to suddenly changed light conditions. Subsequent literature survey revealed striking knowledge-gap of the impact of short-term light-changes (like rainy days) on the mycorrhizal functioning.

Three plant species (medic, leek and ryegrass, Lolium perenne), inoculated with a complex AMF community, were subjected to two soil P levels. C flows were traced by $^{13}$C stable isotope labelling; C allocation to the 16:1ω5 fatty acid served as a proxy of C flow to AMF. Mycorrhizal benefits were lowered by P-fertilization and the C flow to AMF was reduced as well. Importantly, the reduction was independent on the C flow to roots and consist in the reduced amount of the AMF and the reduced C income per unit of AMF as well, suggesting the active role of plant. In addition, fraction of host C budget consumed by AMF (mycorrhizal C cost) was compared to previously published values; it highlights that the C cost of AMF usually fell below 10 %, although higher values are frequently (and sometimes incorrectly) referred in secondary literature.

In conclusion, P flows from AMF flexibly react to sudden light changes which opens the way for AMF to hold a bargain power despite being completely dependent on their host. On the other hand, the decrease of C income of AMF in P-fertilized soil suggests the active suppression of C flow to useless symbionts by the plant host. The thesis highlights the importance of light conditions for mycorrhizal functioning and the unsatisfactory knowledge about the actual C costs of arbuscular mycorrhiza.

Keywords: arbuscular mycorrhiza, costs and benefits, carbon, phosphorus fertilization, light availability, common mycorrhizal networks, stable isotope labelling
3 Abbreviations

AMF – arbuscular mycorrhizal fungus/fungi
MGR – mycorrhizal growth response
CMN – common mycorrhizal network/networks

4 Introduction

A great majority of land plants live in the symbiosis with fungi which colonize both the roots and soil, seeking nutrients and supplying them to the hosts. This coexistence is called mycorrhizal symbiosis or simply the mycorrhiza. It strongly influences plant mineral nutrition and productivity, as well as the carbon and nutrients cycling through the global ecosystem. Since the arbuscular mycorrhiza is the most common type of such symbioses, widespread on the majority of herbs including the most important crops, revealing the principles of its functioning is potentially important not only for the academic knowledge, but also for the understanding of our environment and for human welfare.

4.1 The general principles of mycorrhiza

The world mycorrhiza (Greek: μυκής, mykes – fungus, ρίζα, rhiza – root) was introduced by a German scientist A. B. Frank (Frank, 1885; see also the English translation, Trappe, 2005). He observed and experimentally explored the non-pathogenic fungal colonization of roots, commonly occurred in Prussia on several tree species. Frank correctly inferred that the fungi replaced the root hairs in their role in plant nutrition and that they are fed by their hosts. Thus, the general principles of mycorrhiza were known more than one hundred years ago.

The main characteristic of mycorrhiza is the connection of plant roots with the soil by fungal mycelia (Smith & Read, 2008). The fungi inhabit the host roots without causing usual anti-pathogenic reaction, being fed by plant with the photosynthetically produced organic compounds. The exchange is bidirectional, as the fungi somehow extended the root system of the host plant, supplying plants with mineral nutrients acquired from soil. Fungi could also provide other (non-nutritional) benefits for plant. However, there are some noticeable exceptions from the above-mentioned scheme of the matter exchange – for example, the achlorophyllous plants exploit the fungi (or the environment via the fungi), giving no photoassimilates in return. Mycorrhiza is further characterised by bidirectional signalization and the changes in the plant physiology and anatomy, allowing its establishment and functioning. Several types of mycorrhiza evolved, differing in their symbiotic functions as well as in the involved plant and fungal taxa.

4.2 Arbuscular mycorrhiza and arbuscular mycorrhizal fungi

Arbuscular mycorrhiza (or earlier “vesicular-arbuscular mycorrhiza”) is characterised by an internal colonization of roots by certain microscopic fungi. The symbiosis is
sometimes classed as a type of so-called endomycorrhiza, because the fungi grow both in the inter- and intra-cellular root space, while the surface of the roots remains unchanged by the colonization. But even if the fungal structures are formed inside the cell walls, they never overcome the plasma membrane, staying outside the protoplast. The fungi miss decomposing capacity, thus the symbiosis is employed mainly in the uptake of soil phosphorus. Nevertheless, the distinct characterization of this type of mycorrhiza seems to be taxa of involved fungi (Smith & Read, 2008).

The symbioses is widespread on the majority of land plants (Smith & Read, 2008). The core of its incidence is among herbs – in fact, most of the grasses, legumes or forbs form arbuscular mycorrhiza, including important crops (and model plants) as corn, rice, sugar cane, potato, tobacco, alfalfa or medic. The noticeable exceptions are e.g. the usually non-mycorrhizal families Brassicaceae (including rape and Arabidopsis) and Cyperaceae, or the Orchideace, forming a different type of mycorrhiza. Thus arbuscular mycorrhiza dominates the grassland ecosystems like prairies or savannas. Nonetheless, it is also spread on the many tree species such as apples, maples, palms, thujas or cycads (e.g. Mosse, 1957; Fisher & Jayachandran, 2005; Weber et al., 2005; Midgley et al., 2015). Further, it is also formed by “lower” plants as pterydiophyta or liverworts (Field et al., 2016; Pressel et al., 2016), despite the lack of the true roots in the latter. The important model plant for the arbuscular mycorrhiza became barrel medic (Medicago truncatula) since it has relatively small and “completely” sequenced genome (Young et al., 2011), in addition to the other features as ease of growing or cleistogamy. Another model legume with small genome, Lotus japonicus, was introduced parallel to medic, because it is easily transformable (Stougard, 2014). Furthermore, the mutants with the restricted mycorrhizal colonization (rmc) or with the dysfunctional mycorrhiza are available e.g. for medic or tomato.

In contrast to the wide range of the involved plants, the arbuscular mycorrhiza is formed by members of the single phylogenetic lineage of fungi, which is the phylum/division Glomeromycota (Hibbett et al., 2007; mycobank.org, 21th June 2017), or, if the currently published classification would be confirmed, the subphylum Glomeromycotina (Spatafora et al., 2016). The lineage is highly specialised and relatively little diversified, comprising of less than three hundred described species (Öpik & Davison, 2016; catalogueoflife.org, 29th May 2017), while the estimates of the total number of recent species are only about two or three thousands (Öpik & Davison, 2016). Although the first species of Glomeromycota were described in the half of the nineteenth century (Tulasne & Tulasne, 1845), it took a hundred of years to confirm these fungi as an agent of arbuscular mycorrhizal colonization (Mosse, 1956). Recently, the “complete” genome was sequenced from the same isolate of one species of Glomeromycota (Rhizophagus irregularis) by two independent research groups (Tisserant et al., 2013; Lin et al., 2014).

All known members of Glomeromycota form arbuscular mycorrhizal symbiosis, with the exception of the only one genera (i.e. Geosyphon, living in an obligate symbiosis with cyanobacteria). Arbuscular mycorrhizal fungi (AMF) are obligate biotrophs – they miss the enzymes for the decomposition of the organic matter, gaining virtually all their carbon
from the host plants (Smith & Read, 2008; Kuyper, 2017). With the exception of germinating spores, AMF are unable to live on the artificial media without a living host (Franken, 2010), which makes their cultivation rather challenging. Usually the isolates of AMF (ideally the monosporic isolates) are cultivated in pot cultures together with the host plants (and with a non-defined assemblage of soil microbes). Some isolates were also transferred into the sterile root-organ-cultures on the Petri-dishes.

Mycelia of AMF are aseptate (coenocytic), with the exception of the inactive hyphae, from which the cytoplasm was retracted. They are rather thin – in average, they have a diameter of 3-4 µm, whereas the root hairs are more than 10 µm in diameter (Jakobsen, 1999). The mycelia colonize the roots (intraradical hyphae) and explore the soil (extraradical hyphae). Extraradical (and sometimes also intraradical) hyphae bear spherical spores, which can be used for the morphological identification of AMF taxa. The spores are multinuclear and they are produced asexually. The intraradical mycelia bear several typical structures: Firstly, the highly branching structures, called arbuscules, are produced inside the root cells (normally in cortex). Their extensive surface is surrounded by a cytoplasmic membrane of invaded cell (but the membrane is never penetrated), which offer a large interface for the symbiotic exchange. However, arbuscules have short lifespan as they are regularly degraded after several days of their existence with no harm for their hosting root cell (Alexander et al., 1989). Secondly, the hyphal coils could be produced inside the root cells similarly to the arbuscules. Some AMF species are known to form well-developed arbuscules, while other species form coils, but both structures could be produced by the same fungus and the morphology of colonisation by the same fungal isolate could also depend on the actual host species (Smith et al., 2004). Finally, some species form vesicles, globular structures putatively serving as lipid storage.

Arbuscular mycorrhiza is very ancient – the structures similar to the recent AMF were found in Ordovician and Devonian fossils (Remy et al., 1994; Redecker et al., 2000; Strullu-Derrrien et al., 2014) and it is hypothesised that the symbiosis play a role in the colonization of land by higher plants (Delaux et al., 2015). It is also believed that the plant molecular mechanisms involved in arbuscular mycorrhiza paved the way for the symbiosis between legumes and the nitrogen-fixing bacteria (Parniske, 2008).

4.3 **Nutrient uptake by arbuscular mycorrhiza**

AMF can supply plants with a wide range of mineral nutrients, as phosphorus, nitrogen, sulphur, zinc or copper (Neumann & George, 2010; Giovannetti et al., 2017). However, since AMF miss decomposing capacity, the most important benefit for plant nutrition seems to be the contribution of AMF to the P uptake. That is because plant demands of P are high, but the bioavailability and mobility of this element in the soil solution is usually very low. The dominant soil P forms are inorganic phosphate ions (Pi), H2PO4−, HPO42− and PO43− (Frossard et al., 2011), the anions, which react with the charged surfaces and are strongly adsorbed on soil particles (Hodge, 2017). Thus, the movement of phosphate by mass flow is restricted and the P depletion zones occur around plant roots (Smith &
Read, 2008; Hodge, 2017). AMF could help to overcome these zones, since their mycelia are much thinner and thus “cheaper” than plant roots (see previous section). Moreover, the density of AMF extraradical mycelia in topsoil is counted in metres per gram soil, being higher than the density of roots by several orders of magnitude (Jakobsen, 1999; Leake & Read, 2017). On the other hand, the species of AMF vary broadly in the distance of which the external mycelia expand from roots – it range from no more than 1 cm to 10 (or more) cm (Jansa et al., 2005; Thonar et al., 2011). However, one should keep in mind that the density (and thus distance) of roots in natural systems could be higher than in the experimental setups (Jakobsen, 1999).

AMF take up P from soil solution by H+/Pi symporters, while the proton gradient on the membrane is produced by H-ATPase pumps (Hodge, 2017). Further, the involvement of Na+/Pi symporters is considered (Garcia et al., 2016). The P taken up by AMF hyphae is subsequently transformed to polyphosphate chains, which are transported to the vacuoles and then to intraradical mycelia, where polyphosphate is cleaved so that phosphate is released outside the fungal plasma membrane (Giovannetti et al., 2017). However, the mechanism by which the P is delivered from the fungal cells is unknown (Casieri et al., 2013; Saito & Ezawa, 2017). Arbuscules are the main place of symbiotic P efflux, although they are not the only ones, since mycorrhizal plant P uptake can be functional also in their absence. Plants evolved P transporters specialised on the P uptake from AMF, which are expressed only in the presence of mycorrhiza or at least at much higher levels in the presence of AMF than in their absence (Casieri et al., 2013). For example, there is MtPT4 transporter accompanied with the presence of arbuscules in barrel medic or several transporters (LePT3, LePT4, LePT5) coupled with mycorrhizal colonization in tomato (Requena, 2005; Facelli et al., 2010; Facelli et al., 2014). On the other hand, the presence of mycorrhiza often decreases the expression of those plant P transporters which are involved in the direct P uptake by roots (Courty et al., 2016). The downregulation occurs even if the mycorrhizal P uptake is insufficient to meet plant demands (Grønlund et al., 2013). Direct plant P uptake can be further decreased by the presence of AMF due to the competition between the direct and mycorrhizal P-uptake-pathways (Smith et al., 2011).

The $^{33}$P and $^{32}$P radioisotopes were used to estimate the fraction of plant P which was supplied by AMF. As a result, AMF was found to be responsible for 0-100 % of plant P uptake, with the values distributed rather uniformly along this gradient (Pearson & Jakobsen, 1993; Ravnskov & Jakobsen, 1995; Smith et al., 2004; Poulsen et al., 2005; Li et al., 2006; Grace et al., 2009; Nagy et al., 2009; Facelli et al., 2010; Facelli et al., 2014; Stonor et al., 2014). The measured values vary widely among plant and AMF species as well as experimental set-ups. On the other hand, only six plant species and six AMF species were tested in the named studies. In principle, two types of experimental set-ups were developed. First, a small root-free compartment (i.e. a compartment separated from the bulk soil by a fine mesh allowing hyphae, but not roots, to growth through) was filled by the soil enriched with radioactive P and then buried into the pot before plant sowing. Second, a side root-free compartment was added to the experimental setup during the
plant growth. Such measurement of P flow is challenged not only by the safety issues, but also by the short half-life of $^{33}$P and $^{32}$P isotopes (25 and 14 d, respectively), restricting the age of the plant (and AMF) for few weeks. Another issue is, that the rate of the P uptake from the compartment is influenced by the rate of the growth of extraradical hyphae, i.e. the rate by which AMF are able to penetrate the compartment. Concurrent labelling (and measuring) of P and C flows is challenging too. There was an effort to overcome the safety-risks and time issues associated with P radioisotopes by introducing the stable isotope $^{18}$O bound in phosphate (Frossard et al., 2011). However, the enzymatic activity of organisms and our insufficient knowledge make this approach disputable.

The importance of AMF for plant N nutrition is a matter of an ongoing debate. Although the plant N uptake via AMF was documented many times (e.g. Fellbaum et al., 2012; Weremijewicz et al., 2016), the ecological relevance of this uptake is assumed to be not as high as in the case of P. That is because the NO$_3^-$ ions are highly mobile by mass flows, potentially forming only “shallow” depletion zones (Hodge, 2017). Thus AMF seem to be unable to overcome N-limitation of plant growth (Johnson et al., 2015). However, the increased participation of AMF on the plant N uptake was observed when the importance of NH$_4^+$ as a N source was increased (Hodge & Storer, 2015). The availability of NH$_4^+$ can be restricted since it could be held on the negatively charged substrates, although it is still more mobile than phosphate (Hodge, 2017).

To properly evaluate the potential benefits of arbuscular mycorrhiza for plants one could not omit the non-nutritional benefits such as the potentially improved resistance to the pathogens or drought (Gehring et al., 2017), or the stabilization of soil structure e.g. due to the production of glomalin (Lehmann et al., 2017).

### 4.4 Carbon flow to arbuscular mycorrhizal fungi

Mechanism of C transfer to AMF is still elusive (Casieri et al., 2013; Field et al., 2017). Arbuscules are supposed to be the main (but not the only one) place of the C influx (Casieri et al., 2013; Manck-Götzenberger & Requena, 2016). It has been suggest that sucrose is exported from plant cells, cleaved in the interfacial space and then the monosaccharides are transported into the fungal cells (Doidy et al., 2012). However, this paradigm is now challenged in more than one way – e.g. other organic compounds are suspected to be transferred from plants to AMF.

There are some plant monosaccharide transporters (MSTs) with the expression patterns influenced by the presence of AMF, but none of them showed activation exclusive to mycorrhiza (Casieri et al., 2013; Garcia et al., 2016). Recently, researchers focus on the plant transporter family with beautiful apt name “sugars will eventually be exported transporters” (SWEETs), which can be responsible for both influx and efflux of saccharides to/from protoplast (Manck-Götzenberger & Requena, 2016). None of them was discovered to have mycorrhiza-exclusive activation, but many of them were found to have expression influenced by the presence of AMF (Manck-Götzenberger & Requena, 2016). It is suggested now that the sucrose is both exported to the apoplast (and cleaved
there by cell wall-bound invertases) and cleaved in the cortical cells (and glucose is then exported to apoplast) to supply sugars to AMF (Manck-Götzenberger & Requena, 2016). One MST responsible for the sugar uptake from the symbiotic interface was so far identified in AMF Rhizophagus irregularis (Garcia et al., 2016).

It was said (section 4.2), that AMF gain virtually all their carbon from host plants. And how much of the plant photosynthetic production is consumed by AMF? First experiments dealing with this question were published in early eighties. They use $^{14}$C radioisotopic labelling of plants and various experimental set-ups (e.g. split-roots with one half of the root system inoculated by AMF while the other half remained non-mycorrhizal). These experiments showed that AMF consume 4-10 % of C fixed photosynthetically by their hosts (Kucey & Paul, 1982; Snellgrove et al., 1982; Koch & Johnson, 1984). The later publications showed even higher values – 20 % was found for young cucumber (Jakobsen & Rosendahl, 1990). Despite the subsequent development of the safe $^{13}$C stable isotope labelling and measurement, no boom of the AMF-C-cost research happened, so that we still have only a few publications, working with the limited range of plant and fungal species. All the newer publications show that less than 10 % of plant photosynthetic production is spent by AMF (Johnson et al., 2002b; Grimoldi et al., 2006; Calderón et al., 2012; Slavíková et al., 2017). Some other publications referred about the C transfer to AMF without including the respiration loses, but the amount of C respired by mycelium can be many times higher than the C retained in the mycelium (Lendenmann et al., 2011; Slavíková et al., 2017). Further, all studies about the AMF-C-cost, which included AMF respiration, worked with the single AMF species – with the exception of the imaginative works of Johnson and his co-workers, which studied whole grassland ecosystem and gained the value between 3-6 % (Johnson et al., 2002a; Johnson et al., 2002b).

On the other hand, AMF can increase the photosynthetic production of host plants by various ways (Franken, 2010). Firstly, the improved nutrition of mycorrhizal plants allows the increased amount and activity of photosynthetic tissues (Black et al., 2000). Secondly, AMF sink strength could stimulate photosynthetic activity of plants, or even alleviate the putative sink-strength-limitation of photosynthesis (Kaschuk et al., 2009). Overall, the C flow to AMF could mean no real cost for host plant, if the plant growth is not photosynthetically limited, or if the limitation of the capacity of photosynthetic apparatus could by overcome by the increased nutrient uptake due to mycorrhiza (Tuomi et al., 2001).

4.5 Outcome of arbuscular mycorrhiza – an interplay between partners and environment

It seems that some arbuscular mycorrhizal plants are consistently colonized in field (obligatory mycorrhizal plants), while others were sometimes found without colonization (facultatively mycorrhizal) in nature (Moora, 2014; Bueno et al., 2017). Maybe this can reflect the different ability of plants to survive without AMF or the ability of some plants to suppress AMF under environmental conditions where the mycorrhiza is not needed,
e.g. in highly fertilized soil (Moora, 2014). Nevertheless, the classification of a particular plant species as obligatory/facultatively mycorrhizal might change as empirical data accumulate (Bueno et al., 2017). Moreover, it was argued that non-mycorrhizal state (of normally arbuscular mycorrhizal plant species) in nature is probably caused by the lack of AMF in soil (e.g. after the application of fungicides) and thus the mycorrhizal/non-mycorrhizal state is not optional for plants (Smith et al., 2011).

Most of the plant species which regularly form arbuscular mycorrhiza in field seem not to be principally dependent on AMF, since there is usually no problem to grow them without AMF. Although experimentally grown non-mycorrhizal plants (of species which normally form mycorrhiza) are often smaller than their mycorrhizal counterparts, they are usually able to complete their life cycle. There are also dozens of published experiments where the biomass of non-mycorrhizal plants was higher than that of mycorrhizal plants. The comparison of the biomass of mycorrhizal and non-mycorrhizal plants of the same species grown in the particular conditions is called “mycorrhizal growth response” (MGR). Various calculations are used, e.g. the difference between the biomass (total or shoot) of mycorrhizal and non-mycorrhizal plants, divided by the biomass of non-mycorrhizal plants and expressed in percentages. Similarly, mycorrhizal P response could be calculated using P content of plants or shoots. Nevertheless, the experimental comparison of mycorrhizal and non-mycorrhizal plants could be misleading, since the non-mycorrhizal state may be physiologically artificial for usually mycorrhizal plants (Smith et al., 2011).

The outcome of the arbuscular mycorrhiza for a host plant might be understood as the balance of overall mycorrhizal costs and benefits (Johnson et al., 1997; Johnson, 2010). Thus the term “mycorrhizal phenotype” was introduced to reflect the general outcome of arbuscular mycorrhiza for plant, which may be either positive or negative depending on the environmental conditions as well as plant and fungal genotypes (Johnson et al., 1997). According to this concept, the actual symbiotic functioning lies on the mutualistic-parasitic continuum (in the ecological point of view). Despite the fact that this concept was later assaulted (by one of its authors, among others), e.g. because the physiological functioning of arbuscular mycorrhiza differs from typical infestation by pathogens/parasites (Smith et al., 2011; Smith & Smith, 2012; Smith & Smith, 2013), the model of the mycorrhizal phenotype as an emergent property of plant-fungal interaction remains a useful framework (Johnson & Graham, 2013), producing testable hypothesis (Johnson et al., 2015).

Economy based models of plant resource partitioning suggest that plant should make an effort to reduce C flow to roots and mycorrhiza under the conditions where the growth is not limited by nutrient uptake, e.g. in highly fertilized soil or under light-deprivation (Bloom et al., 1985; Landis & Fraser, 2008; Johnson, 2010; Wyatt et al., 2014). In fact, there are dozens of documented cases of reduced mycorrhizal colonization of plants due to fertilization or decreased light-availability (e.g. Grman, 2012; Johnson et al., 2015; Sochorová et al., 2016; Püschel et al., 2017). Of course, there are also many cases where the colonization was not reduced despite strong P-fertilization or light-deprivation of...
plants (e.g. Hayman, 1974; Tester et al., 1985; Ning & Cumming, 2001; Grman, 2012)
Anyway, the underlying cause of the observed reduced colonization is still disputable. Although the direct action of plants against AMF seems to be logical, some scientists argue that there is still no physiological evidence that AMF are suppressed actively by the plants to avoid the results of disadvantageous symbiotic conditions (Smith et al., 2011; Smith & Smith, 2015).

Paragraphs above deal with the outcome of the arbuscular mycorrhizal symbiosis for the host plants. It is obvious that the benefits from the symbiosis for the fungal partners are infinite since AMF cannot live without plants (Koide & Elliott, 1989). However, this does not mean that they are passive participants in the symbiosis ruled completely by plants. It was observed that AMF Glomus intraradices (=Rhizophagus intraradices) accumulates nutrients when it lives in the root-organ-cultures with reduced sugar availability, in contrast to the situation when the roots were sugar-rich (Bücking & Shachar-Hill, 2005; Hammer et al., 2011). This could indicate that fungus controls P efflux to plant in response to the sugar income and that there is possibly some negotiation in the symbiotic interface so that fungus “waits for better price” under unfavourable exchange condition.

4.6 Partner discrimination and common mycorrhizal networks
The possible negotiation between the host plant and its symbiotic fungi is complicated by the fact that in natural conditions the individual plants are colonized by many individual AMF mycelia and that one individual mycelium can be connected with more plants, forming the so-called common mycorrhizal (or mycelial) network (CMN). Moreover, AMF are not host-specific and vice versa, although some preferences were observed, i.e. that the AMF community structure in roots can differ (to some extent) among host plant species at the same site (e.g. Lekberg & Waller, 2016). The lack of the host specificity leads to the question how can be arbuscular mycorrhizal symbiosis evolutionary stable, i.e. why did it not turn into the classical parasitism if its mutualistic nature is not stabilized by the species-specific coevolution of symbionts? Fitter (2006) speculated, that plant could allocate sugars preferentially to that parts of roots which bring more mineral nutrients so that the nutrient supply from fungus stimulates plant to deliver more sugars – thus, fungi which bring no nutrients (“cheaters”) can still survive in roots, but the more cooperating fungi are favoured.

Furthermore, the sanctioning of non-beneficial partners was considered to stabilise mutualism in arbuscular mycorrhiza in a way that plant could punish non-beneficial fungi either by restricting their colonization or by preferential allocation of C towards better partners, while fungi could preferentially supply more “generous” plants with nutrients (Kiers & van der Heijden, 2006; Kiers & Denison, 2008). There is some experimental evidence supporting the existence of such mechanisms: Firstly, plants might be physiologically able to suppress AMF if no P is gained from it: Arbuscles and intercellular hyphae were degenerated prematurely in Medicago truncatula plants if there were no P-gains from AMF due to dysfunction of mycorrhiza-specific P transporter MtPT4 because of its mutation (mtpt4-1) or silenced expression (by RNA interference in
transformed plants), although the initial states of colonization were normal (Javot et al., 2007).

Secondly, there are experiments indicating that the plants might sanction/reward AMF according to the P supply: If roots in root-organ-cultures had a choice, they supplied C preferentially towards the P-rich mycelia over P-poor mycelia of the same AMF species (Kiers et al., 2011). Nevertheless, this preferential supply appeared only in case of AMF Glomus intraradices (=Rhizophagus intraradices) but not in the case of G. aggregatum (=R. aggregatus) (Kiers et al., 2011). In the same line, two Allium plant species allocate more C to that half of root in a split-root system which was colonized by the more beneficial fungal species – although the preferential allocation failed if the AMF were not spatially separated (Bever et al., 2009). Furthermore, AMF Funneliformis mosseae consumed more C per unit of P supplied to its hosts than R. irregularis if the host plant (Plantago or Trifolium) had no choice between AMF, but if both fungi shared the same host in a split-root system (with each half colonized by one AMF species), F. mosseae changed its behaviour so that both root halves received P for the same C price (Argüello et al., 2016).

Thirdly, there are cases indicating that AMF might sanction/reward plants according to the C supply: Both AMF G. intraradices (=R.intraradices) and G. aggregatum (=R. aggregatus) preferentially supplied P towards the sugar-rich roots over sugar-poor roots in root-organ-cultures (Lekberg et al., 2010; Kiers et al., 2011). Similarly, Fellbaum et al. (2014) found that both AMF R.irregularis and G. aggregatum preferentially supplied fully-lit medics over shaded medics (Medicago truncatula) with both P and N, although the growth of the plants was not significantly influenced by light treatment, which may indicate that the results were not confounded by the different nutrient demands of shaded vs. non-shaded plants. However, actual C flow to AMF was not measured.

Likewise, biological market theory was applied on the cooperative behaviour of microbes including AMF (Werner et al., 2014). It argues that the exchange of goods among organisms can be analysed in market terms, with individuals making strategic investments, under the condition that the members of at least one trader class are able to choose or switch partners. In contrast, it does not assume the cognition of partners since advantageous responses could evolve due to immediate rewards (Werner et al., 2014). Subsequently, the mathematical modelling of resource exchange between plants and AMF revealed that dividing resources among partners in direct relation to the amount of resource received from them can be an evolutionary stable strategy, in an condition that there are enough partners to choose (Wyatt et al., 2014). The market model further predicts the cease of the trade if the P available directly to the plants reaches sufficiently high levels (Wyatt et al., 2014) and the decrease of “price” of P in the symbiotic market under higher P availability (Werner & Kiers, 2015).

However, the importance of the reciprocal rewarding for realized resource exchange in arbuscular mycorrhiza was challenged since the sink-strength of different partners seems to play a role (Walder & van der Heijden, 2015). The extreme example of different sink-
strength can be seedlings sharing CMN with mature plants. However, experiments showed contradictory results: some studies found that the seedlings suffer due to the pre-emption of the soil nutrients by AMF (which supply them preferentially to the larger plants), whereas other studies showed that the growth of the seedling is supported by the presence of CMN connected with adult plants (van der Heijden & Horton, 2009).

Moreover, sink-strength may differ among plants sharing CMN or among AMF sharing the same host not only because of their different demands, but also due to their different ability to exploit their symbionts (Johnson & Graham, 2013; Walder & van der Heijden, 2015). For example, Walder et al. (2012) found that Linum plants received most of the nutrients supplied by AMF in compartmented pot shared with Sorghum plant, although Sorghum plant was the major feeder of AMF, indicating that plant species can receive nutrients from AMF under unequal terms of trade. Furthermore, the extent of inequality in the symbiotic trade differed when different AMF species (Glomus intraradices = Rhizophagus intraradices and G. mosseae = Funneliformis mosseae) were used (Walder et al., 2012). On the other hand, AMF species Claroideoglomus candidum and Gigaspora margarita differed in the amount of C received per unit of P supplied to the host (Allium vineale) which was shared in split-root system with each half colonized by one species (Ji & Bever, 2016).

To summarise, more than one mechanism may play a role in the distribution of photosynthates towards AMF sharing the same host as well as more than one mechanism may play a role in distribution of costs and gains from CMN. This brings interesting insights e.g. on the competition among plants in field: For example, if the nutrients are at least partly distributed among plants according to their C supply to CMN, than the plant competition for light may be translated into the plant competition for mineral nutrients (Weremijewicz & Janos, 2013).

In conclusion, there are many questions about symbiotic functioning of arbuscular mycorrhiza and its impact on the environment which we are not able to fully answered yet, despite the increasing number of experiments focusing on this fascinating topic.

## 5 Aims and hypothesis

This thesis deals with the dynamics of carbon and phosphorus flows in arbuscular mycorrhiza. Besides the thoroughgoing survey of published literature, glasshouse pot experiments were the method of choice to study the symbiotic flows with physiologically intact plants. Both single-species and complex field AMF inocula were used. Carbon flows were traced by the $^{13}$C stable isotope pulse-chase labelling, while the $^{33}$P radioisotope was chosen to trace P flows, based on the preliminary experiments by our Laboratory of Fungal Biology, comparing the usability of $^{33}$P and $^{18}$O-phosphate to trace symbiotic P fluxes (data not published). The thesis focuses on the size and flexibility of C and P flows depending on both abiotic and biotic conditions, in particular on light availability, phosphorus availability and plant species identity. Subsequent objective was
to find out the possible indications that the symbiotic flows are regulated actively by both partners in reaction to the current symbiotic cost and benefits.

Particular aims of this thesis were:

- To assess the effects of duration and intensity of shading on the symbiotic functioning of arbuscular mycorrhiza in a model plant-AMF species combination (publication 1). Specifically, these hypothesis were tested:
  - Both mycorrhizal benefits and the extent of mycorrhizal colonization decline with decreasing light availability.
  - Symbiotic P flow to plants declines rapidly after sudden light-shortage.
  - Plant architecture is changed due to mycorrhizal colonization to achieve higher photosynthetic production.

- To review thoroughly the published literature on the effect of light intensity on the functioning of arbuscular mycorrhiza (publication 2) to reveal:
  - (in)consistence of results among experiments differing in plant and AMF species as well as in abiotic condition,
  - potential knowledge gaps.

- To review our knowledge about carbon flows in mycorrhiza, particularly in arbuscular mycorrhiza (publication 3), with special aim:
  - To summarise existing experimentally based data about carbon cost of AMF (see also publication 4).

- To compare carbon cost of complex AMF community among three plant species using different methods of calculation (publication 4).

- To assess the effects of P-fertilization on the symbiotic functioning of arbuscular mycorrhiza in three plant species combined with complex AMF community (publication 4). Specifically, these hypothesis were tested:
  - Both mycorrhizal benefits and the extent of mycorrhizal colonization are lower in P-fertilized soil.
  - The C flow to AMF is reduced due to P-fertilization.
  - This reduction is more extensive than the reduction of C flow to roots.
  - “Price” of P in symbiotic exchange is lowered by P-fertilization.

Three plant species with different mycorrhizal responsiveness were used to gain some insight as to whether the expected reduction of C flow to AMF is limited to the plants for which the symbiosis is well-balanced.

In addition, abstract of an unpublished experiment is enclosed with the PhD thesis to extend the discussion. The experiment deals with the symbiotic functioning of arbuscular mycorrhiza in the presence of competing host plants, which differ in their light-status.
6 Summary of published and unpublished results

6.1 Publication 1 – Konvalinková et al. (2015), published

Duration and intensity of shade differentially affects mycorrhizal growth- and phosphorus uptake responses of Medicago truncatula.

Konvalinková T1*, Püschel D1,2, Janoušková M1,2, Gryndler M1, Jansa J1
1Laboratory of Fungal Biology, Institute of Microbiology, Academy of Sciences of the Czech Republic, Prague, Czech Republic
2Department of Mycorrhizal Symbioses, Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice, Czech Republic
*Corresponding author


This article presents an experiment focused on the effect of light intensity on the functioning of arbuscular mycorrhizal symbiosis. A model legume plant, barrel medic (Medicago truncatula) was either inoculated or not with an AMF Rhizophagus irregularis and subjected to either long-term (38 d) or short-term (6 d) shading in a glasshouse pot experiment. Four intensities of incoming light were used (100, 65, 35 and 10 %). 13C-isotope pulse labelling was applied in form of gaseous 13CO2 on plants of the most contrasting light treatments (full-light and 10 % long-term) 3 d before harvest to estimate the allocation of recently fixed C between roots and shoots. In addition, harvested shoots flatten by a plexiglass plate were photographed and several morphological traits were assessed by an image analysing software (see appendix 12.3 for illustrative photos).

The long-time shading substantially decreased plant growth. The decrease was non-linear, with the highest drop between 65 and 35 % of the incoming light. Mycorrhizal growth response (MGR) of the plants decreased along the gradient of long-term shading from highly positive in the full-light treatment to negative in 10 % of incoming light. Mycorrhizal colonization of the roots decreased concurrently with the MGR. The plants reacted to the long-term shading by a shoot elongation, decreased root to shoot ratio and increased leaflet surface. Interestingly, the reaction of latter two traits were further boosted by the mycorrhiza, especially in the 35 % level of incoming light, where the plants strongly suffered from the insufficient light but they still benefited from the mycorrhiza (in terms of both growth and P-content). The allocation of recently fixed C to the roots compared to the shoots was lower in 10% of incoming light than in the full-light, and the effect was more pronounced in the mycorrhizal plants. Overall, it seems that the mycorrhizal plants employed several mechanisms to compensate the C/energy demands of AMF under the light deprivation. Possibly, the reduced mycorrhizal colonization might also be a result of a direct plant action, although this needs to be proved. However, the compensatory mechanisms were insufficient under the highest light deprivation.

The short-time shading decreased the shoot biomass independently on the mycorrhizal status of the plants. In contrast, shoot P-content of the mycorrhizal plants declined with
the decreasing light intensity, while the P-content of the non-mycorrhizal plants was unaffected. Root P-content was decreased by the shading only slightly and independently on the mycorrhizal status. This lead to the relative accumulation of P in the roots at the expense of the shoots in the shaded mycorrhizal plants, visible also as the increased root P-concentration. In another words, while the non-mycorrhizal plants continued in P uptake from soil to the shoots after the sudden shading, the mycorrhizal plants showed the decreased P transfer to the shoots. Because P is normally redistributed among plant organs to meet their needs, the P accumulated in roots under the light shortage was probably retained in the fungal tissues.

In conclusion, the most important findings of this work are:

- Morphological adaptation of barrel medic to the long-term light deprivation boosted by the symbiosis with AMF *Rhizophagus irregularis* (probably because of C demand of the fungus and due to the improved nutrition of the mycorrhizal plants). To the knowledge of the authors, this is the first documented evidence of the morphological adaptation to low light boosted by AMF.
- Accumulation of P in the roots of mycorrhizal plants under the sudden light shortage, while P in the shoots declined. This may indicate that the AMF continued to take up P from the soil but did not supply it to the plant, probably in reaction of decreased C supply from the hosts.

### 6.1.1 Statement of contribution

The PhD candidate and other authors jointly devised the structure of the experiment. The candidate carried out the experiment and most of the sample analyses, with the substantial assistance of the other members of the Institute of Microbiology and the Institute of Botany. The PhD candidate analysed the data and summarised the results. The candidate and the other authors jointly wrote the manuscript and contributed to the revisions.

### 6.2 Publication 2 – Konvalinková and Jansa (2016), published

**Lights off for arbuscular mycorrhiza: On its symbiotic functioning under light deprivation.**

**Konvalinková T**\(^1, 2\) and Jansa J\(^1\)*

\(^1\)Laboratory of Fungal Biology, Institute of Microbiology, The Czech Academy of Sciences, Prague, Czech Republic  
\(^2\)Department of Experimental Plant Biology, Faculty of Science, Charles University in Prague, Prague, Czech Republic  
*Corresponding author


Based on the results of publication 1, this article thoroughly reviews available literature about the effect of light intensity on the symbiotic functioning of arbuscular mycorrhiza. It shows a great variability in the results of experiments involving different plants, AMF and light treatments. Mycorrhizal growth response (MGR) was often decreased by low light intensity, but there is almost equal number of published experiments with no
observed effect of light treatment on MGR despite the treatment strong enough to reduce plant growth. The same is true for mycorrhizal colonization of roots. The causes of decreased MGR under low light availability, i.e. C demand of AMF and inability of AMF to supply enough P with low C income accompanied with suppressed direct plant P uptake, are discussed, together with the evidence for the active reaction of both partners on the changed symbiotic conditions.

The most important findings are:

- The importance of light availability for mycorrhizal functioning.
- Striking knowledge-gap of the impact of sudden light-changing events (like rainy days) on the mycorrhizal functioning, emphasising the importance of research focused on the rate of partners reactions to these changes.

6.2.1 Statement of contribution

Both PhD candidate and the other author devised the structure and decided on the content of the paper, the candidate conducted the literature survey, and then both authors jointly wrote the manuscript and contributed to revisions.

6.3 Publication 3 – Řezáčová et al. (2017a), in press

<table>
<thead>
<tr>
<th>Carbon fluxes in mycorrhizal plants.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Řezáčová V¹, Konvalinková T¹, Jansa J¹*</td>
</tr>
</tbody>
</table>

¹Laboratory of Fungal Biology, Institute of Microbiology, The Czech Academy of Sciences, Prague, Czech Republic
*Corresponding author


This chapter reviewed the knowledge about the magnitude of C flow to mycorrhizal fungi and its impact on the ecosystem functioning, being focused particularly on the arbuscular mycorrhiza. The published experiments revealing the C cost of AMF are summarised, highlighting the importance of respiration measurement for the C cost estimations. The values are compared with those observed in other mycorrhizal types, and the lack of information about the magnitude of C flow in the ericoid mycorrhiza is acknowledged. The molecular mechanisms of the C flow to the mycorrhizal fungi are reviewed briefly, with respect to the fragmentary character of current knowledge. The further ecological impact of C gained by the mycorrhizal fungi is discussed in short, including e.g. the redistribution of symbiotic costs and benefits in common mycorrhizal networks, the feeding of “hypsymbiotic” microbes on fungal exudates or the increased stability of soil aggregates due to the production of glomalin.
The most important finding is that the experimental estimations of C cost of AMF most often fell below the 10 % of total plant C budget, although the higher values are frequently cited in literature in summarising statements.

6.3.1 Statement of contribution
The PhD candidate conducted the initial summarisation of literature on the C cost of arbuscular mycorrhizal symbiosis and the C budget of arbuscular mycorrhizal plants, and then also contributed to writing of the manuscript.

6.4 Publication 4 – Konvalinková et al., submitted

Carbon flow from plant to arbuscular mycorrhizal fungi is reduced under phosphorus fertilization

Konvalinková T¹,²,*, Püschel D², Řezáčová V², Gryndlerová H², Jansa J²

¹Department of Experimental Plant Biology, Faculty of Science, Charles University in Prague, Prague, Czech Republic
²Laboratory of Fungal Biology, Institute of Microbiology, The Czech Academy of Sciences, Prague, Czech Republic
*Corresponding author

Original research article, submitted to the Plant and Soil. The version after second round of revision is enclosed.

This manuscript presents a glasshouse experiment focused on the effect of initial P-fertilization on the C and P flows in arbuscular mycorrhiza. Three plant species, leek (Allium porrum), barrel medic (Medicago truncatula) and ryegrass (Lolium perenne), were inoculated with field-soil microbial inocula with/without native AMF and subjected to two soil P-levels (with/without additional P). The P flow to plants from the symbiotic mycelia was traced by the ³²P radio-isotope, applied to the root-free compartments 8 d before harvest. The ¹³C-isotope labelling was applied 5 d before harvest. Then the belowground respiration was continuously collected from the low-P-pots, while the dark respiration of shoots was estimated from its collection at the first night, to gain whole C budget of plant. In addition, the C flow to AMF was traced down to the AMF-signature fatty acid 16:1ω5 in roots. Various methods of estimation of the mycorrhizal C cost were applied.

Mycorrhizal growth response was positive in leek, neutral in medic and negative in ryegrass, with no effect of the fertilization. Mycorrhizal P response was positive in leek and medic and neutral in ryegrass in the low-P-pots and it was considerably decreased by the P-fertilization. Mycorrhizal colonization of roots was decreased by P-fertilization in leek and ryegrass. The mycorrhizal plants allocated higher portion of the recently fixed C to the belowground respiration than the non-mycorrhizal plants, although the C allocation to the belowground in general was not influenced by the AMF. The mycorrhizal C cost for medic ranged 2.6-10.5 % of the plant C budget, depending on the method of calculation; only one method was applicable for leek and another for ryegrass, retrieving values 0.9 and 6.2 %, respectively.
The C flows to AMF, both per g root and in the whole root system, were decreased by P-fertilization. However, looking at each plant species separately, the decrease in C flow to AMF per g root by P-fertilization was significant in leek and ryegrass, but not in medic (despite nonsignificant interaction of plant species and P-fertilization). Importantly, the ratio of the C flow to AMF to the C flow to roots was also decreased by the P-fertilization in leek and ryegrass, while the ratio of root to shoot C allocation was unchanged. In another words, the C flow was suppressed specifically towards the AMF due to P-fertilization, i.e. independently of the C flow to roots. Analysis of the individual components forming the C flow to AMF revealed, that not only the mass of AMF, but also their relative C income was decreased under P-fertilization.

In addition, we aimed to compare the ratio of C allocated to AMF per unit of $^{33}$P supplied by AMF to the host plants between the two P-fertilization treatments. However, we were able to quantify the P uptake by AMF only in the medic plants (because of wide discrepancies in P-uptake from labelling compartments by leeks within particular treatments and a high P-uptake from root-free compartments by non-mycorrhizal ryegrass) and the ratio of C allocated to AMF per unit of $^{33}$P supplied by AMF was not influenced by P-fertilization.

Based on this results, the most important findings are:

- The reduction of C flow to AMF by initial P-fertilization in leek and ryegrass.
- The specificity of this reduction to AMF (i.e. it was not caused by the general reduction of C flow to roots). To the knowledge of authors, this is the first documented case where the independence of these two flows in their response to P fertilization was tested.
- The composition of this reduction forming not only by the reduced amount of the AMF in the roots but also by the reduced C income per unit of AMF, suggesting (together with the previous finding) the possibly active role of plant.
- The mycorrhizal C cost ranging 0.9-10.5 % (in accordance with the literature survey and in contrast to the often cited values, see publication 3), depending strongly on the method used.

### 6.4.1 Statement of contribution

The PhD candidate and the other authors jointly devised the structure of the experiment. The candidate carried out the experiment and most of the sample analyses, with the substantial assistance of the other members of the Institute of Microbiology. The PhD candidate analysed the data, summarised the results and wrote the first version of the manuscript. Then the candidate and the other authors jointly contributed to the writing of the subsequent versions of the manuscript and contributed to the revisions.
6.5 Abstract of an unpublished experiment

Fluxes of carbon and phosphorus in common mycorrhizal network – who is fed by arbuscular mycorrhizal fungi and who makes the payment?

Konvalinková T¹, ², Püschel D², Řezáčová V², Gryndlerová H², Slavíková R², Hujšlová M², Konečný J², Bukovská P², Gryndler M², Jansa J² #

¹Department of Experimental Plant Biology, Faculty of Science, Charles University in Prague, Prague, Czech Republic
²Laboratory of Fungal Biology, Institute of Microbiology, The Czech Academy of Sciences, Prague, Czech Republic
#Preliminary list of authors.

Original research, presented in part on the 8th International Conference on Mycorrhiza (Flagstaff, Arizona, 2015; lightning talk) and on the ISME Meeting of Young Soil Microbial Ecologists (Prague, 2017; talk). The PhD candidate was the presenting author in both events.

Only short summary of the experiment is enclosed with the PhD thesis, consisting of the abstract from the second conference (after some grammar corrections), supplemented with the figures and comments to the main results, because the full manuscript is not ready yet. However, the results are believed to be valuable to discuss, although the candidate acknowledges the importance of the full data for the proper discussion.

This is a short presentation of the experiment focused on the C flows to the AMF and P flows from the AMF to the competing plants. Two plant populations shared the same pot, competing conspecifically or interspecifically (Medicago truncatula, Allium porrum) in the presence or absence of the AMF. One, both or none of the plant populations was shaded during the last 5 days before the harvest. The shading was applied concurrently with the ¹³C and ³³P isotopic labelling.

The conspecific plant P-uptake competition was intensified in the presence of AMF in favour of the fully-lit plants (i.e. at the expense of the shaded plants). The interspecific competition between the medic and the leek was also intensified by the presence of AMF, when both plants received full light. However, the dominance of the medic over the leek in the current plant P-uptake was lowered, when the medic was shaded (with no respect to the light-status of the leek), but only in the presence of AMF. Values of the recently fixed C allocated to the AMF-signature fatty acid 16:1ω5 varied broadly within the particular treatments. Nevertheless, there was a significantly lesser allocation of recently fixed C to the AMF by the shaded medic than by the fully-lit medics.

To conclude, the main findings are:

- Fully-lit plants favoured in P-uptake over the shaded plants due to AMF, maybe because of preferential allocation of P to the fully-lit plants by AMF.
- No mycorrhizal benefits in P-uptake for leek in the presence of competing medic until the medic was suddenly shaded, followed by the quick redirection of
mycorrhizal P-benefits towards the leek, indicating a large plasticity in the mycorrhizal P flows.

6.5.1 Statement of contribution
The PhD candidate and the other authors jointly devised the structure of the experiment. The candidate carried out the experiment and most of the sample analyses, with the substantial assistance of the other members of the Institute of Microbiology. The PhD candidate analysed the data and summarised the results.

7 Discussion
Rather than discussing thoroughly all topics connected to this PhD thesis, this section focuses only on several aspects of the research to avoid undue overlapping with the discussion sections of the enclosed publications.

7.1 Light availability as a basis of symbiotic functioning of arbuscular mycorrhiza
It is anticipated that plants change resource allocation after shading to prefer shoots over the roots and mycorrhizas (Johnson, 2010). Since light is the basic source of energy not only for plants but also for AMF, the efficiency of symbiotic functioning should be lowered subsequently (Johnson et al., 1997). As expected, both mycorrhizal growth response and mycorrhizal P response of Medicago truncatula on Rhizophagus irregularis were lowered by shading in our experiment (Konvalinková et al., 2015), as it was shown previously for many other plant and fungal species combinations (see Konvalinková & Jansa, 2016 for a review). Nevertheless, two features which were rarely used in previous experiments – the usage of four-point gradient of light intensity and the application of sudden short-time shading – allowed us to gain a deeper insight into the symbiotic functioning of arbuscular mycorrhiza under various light conditions.

7.1.1 Plants adapt themselves to C sink of AMF
Arbuscular mycorrhizal fungi may consume substantial part of organic compounds produced by their hosts (see section 7.2). Plants adapt to the additional C sink presented by symbiotic fungi in various ways: Increased rates of photosynthesis (Wright et al., 1998b; Kaschuk et al., 2009; Franken, 2010; Johnson et al., 2015), decreased root-to-shoot ratio or other changes in plant architecture to support photosynthetic production (Bethlenfalvay & Pacovsky, 1983; Harris et al., 1985; Wright et al., 1998b; Miranda et al., 2011; Zhang et al., 2016) were observed in reaction on mycorrhiza. It was argued that if the extension of root system by mycelia of AMF allows plants to have less roots, than the C sink of AMF might be lower than C sink of “additional” roots of non-mycorrhizal plants (Johnson et al., 1997). However, one should not forget that AMF spend resources not only for searching nutrients for plants but also for their own needs (as their own
reproduction) and that the energetic losses across trophic levels are high (Tinker et al., 1994; Neumann & George, 2010).

We did not measure plant photosynthetic rate in our experiment (Konvalinková et al., 2015), however, we observed changes in plant morphology caused by the presence of AMF. Apart from morphological traits which were changed obviously due to increased growth/development of mycorrhizal plants (number of shoot branches and length of the main axis), we had identified two other traits reflecting changes in plant resource partitioning due to mycorrhiza: root-to-shoot biomass ratio and average leaflet surface. Interestingly, none of them was influenced by mycorrhiza at full-light, where the incoming light was not the limiting factor for plant growth, but both were influenced by AMF under the 35 % of incoming light applied over long-term, where plants were apparently light-deprived. This documented how flexibly morphology of some plants may react on carbon/energy demands of symbiotic fungi along the gradient of light availability. To the best of the knowledge of all co-authors, this was the first documented case where changes in plant morphology caused by insufficient irradiance were further increased by the presence of AMF (Konvalinková et al., 2015). However, there were no differences in morphological traits of mycorrhizal and non-mycorrhizal plants under the 10 % of incoming light, where growth depression by mycorrhiza was apparent. This might document threshold-limitation of both plant acclimatization and mycorrhizal cooperativeness (Landis & Fraser, 2008; Johnson, 2010; Grman et al., 2012; Wyatt et al., 2014).

7.1.2 How fast are changes in symbiotic flows after sudden light-shortage?

Fungal growth in roots can be reduced in light-deprived plants, although we do not know whether it is a result of direct action of plant against the AMF or whether it is caused passively by AMF starving since less assimilates are available in roots in general (see Konvalinková & Jansa, 2016 for a review). For example, Tester et al. (1986) found decreased rate of initiation of mycorrhizal infection in Trifolium plants grown in low-light condition. However, what happens if the well-established plant-fungus symbiosis is exposed to the sudden energy-shortage? It was shown that mycorrhizal colonization of roots might increase as early as 4 days after the initiation of mite herbivory on Lotus leaves due to increased nutrient demands of the plants (Nishida et al., 2009). However, shading represents different problem for plants than defoliation, since their photosynthetic tissues are not damaged and so the nutrient demands are not so pressing. There was only a few experiments dealing with abrupt light-shortage (Konvalinková & Jansa, 2016), although it is obvious that the plant photosynthetic rate will decline immediately after the shading event and then prompt changes in plant resource policy are expected.

We did not find any changes in the extent of mycorrhizal colonization in roots of Medicago due to sudden short-time shading in our experiment but we found decreased P-uptake benefits from mycorrhiza accompanied with the accumulation of P in roots as early as 6 days after the application of shading (Konvalinková et al., 2015). Similarly, there was no effect of 5-day-shading on the hyphal root colonization of medic or leek by
complex AMF community in our unpublished experiment (sections 6.5 and 12.9, data not shown), but there were significant shifts in mycorrhizal P uptake benefits for plants caused by the shading (section 12.9, Fig. 4 and 6), accompanied with changes in the abundance of arbuscules in case of the leek (only in interspecific competition treatment, p=0.016, data not shown). Furthermore, significantly higher fraction of plant C budget was delivered to the AMF-signature fatty acid 16:1ω5 by fully-lit medics than by shaded medics (section 12.9), although this was most probably caused by general reduction of C allocation to roots (p<0.001, data not shown), rather than by specific suppression of C flow to AMF.

Likewise, Fellbaum et al. (2014) found higher AMF-driven P uptake (from root-free compartments) by fully-lit vs. shaded medics 6 days after the application of shading. The difference was apparent for either R. irregularis- or G. aggregatum-inoculated pots shared by both fully-lit and shaded plants, but also for R. irregularis-inoculated controls where both plants in one pot received same irradiance. Mycorrhizal P transfer to host plants correlated strongly with the expression of mycorrhiza-specific plant P transporter MtPT4, thus its expression was also decreased by 6-day-shading (Fellbaum et al., 2014). Furthermore, Konečný et al. (unpublished data) found decreased expression of MtPT4 just after 3 days of sudden shading. In addition, some mycorrhiza-induced plant genes related to sugar metabolism showed decreased expression after 3-day-shading (Konečný et al., unpublished data), promising new insights into the dynamics of symbiotic flows in arbuscular mycorrhiza.

To summarise, although light is the basic source of energy for both plants and AMF so that rapid changes in plant resource demands and allocation are expected after sudden shading, only few studies focused on its immediate impact on the symbiotic functioning of mycorrhiza. We do not know how quickly is symbiotic exchange rearranged after shading, let alone which molecular mechanism is influenced first or how quickly is “the trade” restored after return of light (or after acclimatization of symbionts), despite the frequency of sudden light-shortages such as rainy days or predictable light-shortages such as nights in nature.

7.1.3 What can AMF do if their C income is lowered?

What can and AMF do if its host reduces symbiotic flows e.g. because of insufficient irradiance? Published experiments concerning root-organ cultures have shown, that AMF G. intraradices accumulates mineral nutrients instead off supplying them to roots if sugar availability to the roots was reduced (Bücking & Shachar-Hill, 2005; Hammer et al., 2011). This nutrient retaining might represent a “negotiation” strategy of fungus to achieve more C from host (Grman et al., 2012). Our experiment (Konvalinková et al., 2015) proved the retaining of phosphorus by AMF in reaction to the sudden energy-shortage in more realistic model with complete intact plants, Medicago truncatula. In combination with the fact that plant P transporters for direct root P uptake can be downregulated after mycorrhizal colonization irrespectively of the plant P status
(Grønlund et al., 2013), our finding supports the idea that AMF hold a bargain power, despite being fully dependent on their host.

Since the mycelia of AMF are known to form common mycorrhizal networks (CMN) connecting more than one plant hosts, another strategy for starving AMF could be to redirect their sources to a more “generous” host. In nature, the photosynthetic capacity of connected plants may vary not only due to events affecting whole community (as weather changes), but also individually, e.g. due to selective herbivory or overgrowing of one plant by the other (Fig. 1). It was shown in root-organ cultures that AMF G. intraradices and G. aggregatum supply P preferentially to the C-rich roots (Lekberg et al., 2010; Kiers et al., 2011). An experiment with compartmented pots showed that substantial amount of nitrogen in the shoots of Andropogon gerardii was transferred by CMN (mixture of several AMF species) from the soil in immediate vicinity of neighbouring plants, but only if the target plants was not shaded (Weremijewicz et al., 2016). However, it was impossible to distinguish whether the observed difference was caused by reciprocal behaviour of AMF or whether it was caused by higher N-sink strength of fully-lit target plants compared to the shaded ones (Weremijewicz et al., 2016). Similarly, R. irregularis increased the growth of fully-lit medics more than the growth of shaded medics in the experiment where both plants shared compartmented pot, despite the lack of difference in growth of fully-lit vs. shaded medics in non-mycorrhizal control (Knegt et al., 2016). Analogous experiment with medic inoculated by R. irregularis or G. aggregatum clearly showed preferential allocation of both P and N by AMF from root-free compartments to the fully-lit plants (Fellbaum et al., 2014). However, none of those experiments actually traced C flows to AMF.

Our unpublished experiment (sections 6.5 and 12.9) differs substantially from the previous ones in more aspects: Firstly, complex field soil AMF inoculum was used to reveal how would preferential allocation of nutrients be manifested in hosts which are colonized by multiple fungal species, differing in their abilities and life-history strategies (Ijdo et al., 2010). Secondly, roots of both shaded and non-shaded plants shared the same soil volume so that it was easier for fungi to be connected to different hosts and direct root competition for nutrients was not restricted as well – like in field conditions. Finally, plants of two species were combined and their C-status was reversely manipulated to reveal whether the fungal choice between two host species could be changed by light conditions. In conspecific competition, both fully-lit medics and leeks were preferred by

![Fig. 1: The caricature of plants joining CMN but experiencing different light condition. Drawing by T. Konvalinková, colours by I. Michal.](image-url)
AMF in P supply (section 12.9, Fig. 4), although decreased C allocation to AMF due to shading was found only in medic. Interspecific plant competition was strongly dominated by medic so that leek was unable to benefit from mycorrhiza at full light. However, sudden shading of medic induced prompt redirection of mycorrhizal P uptake benefits to leek (with no respect to its own light-status; section 12.9, Fig. 6). This illustrates the flexibility of mycorrhizal P flows in CMN since the allocation between two plant species was modified immediately after the change of light conditions. Nevertheless, we were unable to distinguish unequivocally whether the observed dynamics in P partitioning resulted from fungal choice based on plant C supply or whether it was caused by decreased nutrient sink-strength of shaded medic.

Looking at such experiments, one should still keep in mind that not all individual mycelia in one experimental pot (or in field) are necessarily connected to the all plants (i.e. not all individual AMF really have a choice). Explorative growth pattern of extraradical mycelia, characterised by lower degree of branching, was observed under low-C conditions compared to high-C conditions in root-organ cultures with R. irregularis, which may indicate fungal foraging for new host (Olsson et al., 2014). Since AMF are energetically dependent on the organic compounds from their hosts, it is expectable, that their spore production would decrease due to lower C-income of their host, as was observed either in root-organ-cultures with low-C media or in whole plant models after defoliation (Daft & Elgiahmi, 1978; Ijdo et al., 2010; Olsson et al., 2014). However, there are also cases where production of AMF spores or vesicles increased after defoliation (Klironomos et al., 2004), which may represent a fungal strategy “to wait” for a more suitable host.

7.1.4 Importance of sufficient light availability for studies on plant symbiotic interactions

Irrespective of whether the P flows in arbuscular mycorrhiza are directed more by fungal choices or by nutrient sink-strength of host plants in natural conditions, this thesis highlights the serious impact of light availability on mycorrhizal functioning (Konvalinková et al., 2015; Konvalinková & Jansa, 2016; unpublished experiment, section 12.9). It is essential therefore to pay attention to the sufficient lighting of plants when studying their symbiotic interactions. Although the controlled environments such as growth chambers are advantageous (or even necessary) for many research areas, they usually supply only a fraction of natural irradiance, so that the potential impact of light-lmitation of plants must be considered. The Medicago truncatula Handbook recommend to avoid PAR values lower than 300 µmol m\(^{-2}\) s\(^{-1}\) in experiments with this model plant (Barker et al., 2006). This fits in with sharp changes in plant morphology and morphological responses on mycorrhiza between 65 and 35 % of incoming light in our experiment (Konvalinková et al., 2015).

Interestingly, morphology-devoted section of the same handbook described M. truncatula leaflets as “hairy, sometimes with a dark spot in the centre” (Moreau, 2006). The occurrence of anthocyanin spots depends on the cultivar of medic and it was ignored by publications dedicated to the standardised description of medic shoots development.
(Bucciarelli et al., 2006; Moreau et al., 2006). However, it was absolutely regular in all of our experiments with the commonly used medic cultivar Jemalong, unless the light-intensity was decreased to the levels where substantial changes in plant growth, morphology and symbiotic functioning were apparent (see Fig. A2 in section 12.3). Possibly, the researchers might use the occurrence of leaflet spots in this medic cultivar as an indicator for the appropriate light conditions in their experiments on symbiosis.

7.2 Carbon cost of arbuscular mycorrhiza – what do we really know?
It is difficult to experimentally assess the amount of photosynthetically fixed C which is delivered to the AMF, despite the fact that AMF gain virtually all their C from host (Řezáčová et al., 2017a). That is, besides other reasons, because it is difficult to separate C consumed by root- and fungal cells in mycorrhizal roots or C consumed by AMF and other microbes in bulk soil. The comparison of mycorrhizal plants and their non-mycorrhizal counterparts may be misleading, since non-mycorrhizal state might be highly artificial for usually mycorrhizal plant species, influencing their physiology in unpredictable ways (Smith et al., 2011). Not only for this reason, mycorrhizal plants may allocate less C to belowground than non-mycorrhizal plants, either absolutely or per unit of roots, which makes the classical calculation of AMF cost impossible, as it happened with leek and ryegrass in our experiment (publication 4, sections 6.4 and 12.7). Such problems can be at least partially overcome with split-root plants with half of the root system staying non-mycorrhizal (Koch & Johnson, 1984; Douds et al., 1988) or by using ingrowth soil-cores, in part root-free and in part without AMF mycelia due to regular rotation (Johnson et al., 2002a; Johnson et al., 2002b). Another issue is that diverse method of calculation of mycorrhizal C cost may vary considerably in the results, as appeared in our medic plants (section 12.7, table 2).

It is possible to directly measure the C retained in extraradical hyphae (Jakobsen & Rosendahl, 1990) or to estimate this value from the C retained in signature fatty acids (Olsson & Johnson, 2005; Olsson et al., 2005), however, it is also important to know how much C is respired by AMF (Nottingham et al., 2013). Our knowledge about the ratio of C incorporated in hyphae per C consumed by AMF is limited: values between 0.04 and 0.51 were reported (Kucey & Paul, 1982; Harris et al., 1985; Jakobsen & Rosendahl, 1990; Grimoldi et al., 2006) and we could assume that the real C-use efficiency varies considerably due to environmental conditions as well as internal factors as fungal life-cycle. Mycelia of AMF are characterised by rapid turnover – the average lifespan of extraradical hyphae was estimated as 5-6 days (Staddon et al., 2003). The flux from plant leaves to AMF and from them to atmosphere is also prompt – substantial amounts of CO₂ accounted to AMF are respired in less than 2 days after the photosynthetic fixation (Eissenstat et al., 1993; Lendenmann et al., 2011; Calderón et al., 2012; Slavíková et al., 2017). Our experiment illustrated another aspect of respiration measurement: although there was substantially lower level of mycorrhizal root colonization as well as C incorporation into the intraradical mycelia in ryegrass than in medic, the estimation of mycorrhizal carbon cost based on respiration was higher for ryegrass than for medic.
For this reasons, respiration loses should be measured directly when assessing total C flow to arbuscular mycorrhiza.

The published values of mycorrhizal C cost which somehow dealt with AMF respiration (or used extremely short chase-period) are summarised in this thesis (Řezáčová et al., 2017a; publication 4, section 12.7, table 1). Most of them were gained only for a single plant combined with a single fungal species (but see Wright et al., 1998a; Johnson et al., 2002a; Johnson et al., 2002b). By contrast, our research brings new values for complex field AMF community (publication 4, section 12.7, table 2), since the complexity of AMF inoculum in experiments was found to be important for the outcome of arbuscular mycorrhiza for plants (Hoeksema et al., 2010). Despite all the cautions and uncertainty in the estimations of mycorrhizal C cost, this thesis highlights that the measured values usually fell deep below 10 % of plant photosynthetic production. The maximal published value (20 %) was gained only for 3-weeks old cucumber (Jakobsen & Rosendahl, 1990). This value is often used to summarise the cost of AMF as “up to 20 %” of plant assimilates. This correct but slightly unbalanced statement could be found not only in introductions of research articles on particular aspects of plant-soil C flows (e.g. Gamper et al., 2005; Drigo et al., 2010; Piippo et al., 2011), but also in the secondary literature (Parniske, 2008; Franken, 2010). Moreover, 10 % as a lower limit of AMF cost is sometimes cited in reviews focused on mycorrhiza or directly on the symbiotic C flows (Valentine et al., 2013; van der Heijden et al., 2015; Walder & van der Heijden, 2015).

Particular attention should be paid to the publication by Drigo et al. (2010) which referred to the experiment with Festuca rubra (and naturally non-mycorrhizal Carex arenaria as a control): “In our study, the total incorporation of plant photosynthetates into mycorrhizal fungi was up to 30 % of the total fixed $^{13}$C in F. rubra rhizosphere soil at ambient atmospheric CO$_2$ and up to 40 % at elevated CO$_2$”. In this case, the 100 % means $^{13}$C retained “in F. rubra rhizosphere”, i.e. neither respiration nor $^{13}$C in shoots is taken into account (see Fig. S4 in Drigo et al., 2010). However, some researchers were confused at times, so that this publication is sometimes incorrectly cited as the maximal value for the fraction of photosynthetic production consumed by AMF (e.g. Schnoor et al., 2011; Walder & van der Heijden, 2015).

In conclusion, this thesis points out that our knowledge about carbon cost of arbuscular mycorrhiza is still limited, despite the widespread nature of the symbiosis appealing to incorporate it in global biosphere models (Brzostek et al., 2017). Although the amount of C consumed by AMF is surely not negligible, scientific awareness about it might be confused by either unbalanced or misinterpreting citations.

### 7.3 Arbuscular mycorrhiza under P-fertilization

It is anticipated that the importance of mycorrhizal P uptake for plants decreases with increasing soil P fertility (Johnson, 2010) so that the volume of P-for-C “trade” between symbionts decreases and AMF lose fitness subsequently (Wyatt et al., 2014). Furthermore, the amount of C allocated to AMF per unit of P gained from them by plant
should decrease with increasing P availability (Werner & Kiers, 2015), although this was probably never tested. On the other hand, experiments with wheat and tomato showed that the fraction of plant P which came from AMF symbiont (*G. intraradices*) is lowered in P-fertilized soil (Li *et al.*, 2006; Nagy *et al.*, 2009). Concurrent decrease of both mycorrhizal benefits for plants and mycorrhizal colonization due to P-fertilization was documented many times (e.g. Grman, 2012; Johnson *et al.*, 2015; Püschel *et al.*, 2017; Řezáčová *et al.*, 2017b). Nonetheless, it was argued that there is still no unequivocal evidence for direct coupling of C flow to AMF with P flows from AMF to plants (Smith & Smith, 2015; Walder & van der Heijden, 2015), although it was shown that if the plant P uptake from the symbiotic interface is blocked, arbuscules are degenerated prematurely, despite the normal initial development of colonization (Javot *et al.*, 2007).

In our experiment, both mycorrhizal colonization of roots and mycorrhizal P uptake response of plants were decreased by P-fertilization, although mycorrhizal growth response (MGR) was not affected (publication 4, sections 6.4, 12.7 and 12.8). Three plant species (leek, medic and ryegrass) were used, differing broadly in their MGR. The results with ryegrass are of particular interest as its P content was decreased significantly by mycorrhiza in P-fertilized soil, despite very low level of mycorrhizal colonization in that treatment. Similar pattern was observed previously for another grass species, non-native *Panicum bisulcatum*, grown in the same conditions and it was accounted to the mycorrhiza-induced decrease of direct plant P uptake (Řezáčová *et al.*, 2017b).

The direct measurement of P flow from AMF failed in our experiment in the case of two out of three tested plant species (see Supplement for publication 4, section 12.8). Thus the hypothesis of devalued P against C under P-fertilization was only tested for medic and it was not confirmed in that case.

Considering the decreased levels of mycorrhizal colonization in P-fertilized soil in our experiment (publication 4, sections 6.4 and 12.7), it is not surprising that C flow to AMF, traced to the AMF-signature neutral lipid fatty acid (NLFA) by the $^{13}$C isotope label, was also lowered under P-fertilization. Similar observation was reported by Olsson *et al.* (2010) for *Trifolium subterraneum* inoculated with a single AMF species (*G. intraradices*). In that case, the decrease of C flow to AMF was apparent just one week after P-addition to the pots with established mycorrhizal plants. The question arise whether the reduced colonization and C flow to AMF in P-fertilized soils is caused actively by plant, either directly by restricting C flow to AMF or indirectly by supressing fungal growth and activity in another way.

Our results differ from that by Olsson *et al.* (2010) in an important aspect: While the reduced C flow from *Trifolium* to the AMF-signature NLFA was caused by the reduced amount of this NLFA in P-fertilized treatments (Olsson *et al.*, 2010), we have observed that the reduced C flow to AMF from both leek and ryegrass under P fertilization was caused by reduced amount of AMF-signature NLFA and its reduced $^{13}$C-enrichment as well. In another words, not only the mass of AMF, but also their relative C income was reduced under P-fertilization. However, we should not forgot that the C flow to NLFA
might be misleading for such interpretations since it reports about C stored in fungal lipids, while respiration loses are not accounted for. Thus the decreased $^{13}$C-enrichment of NLFA might mean the reduced C-sink activity of AMF in general or the slowed down allocation of recent C specifically to AMF lipid structures as well. The reduced spore formation at the expense of branched absorbing structures was observed on extraradical hyphae of *R. irregularis* (formerly *G. intraradices*) under elevated P availability, which was interpreted as a strategic shift from reproduction/storage towards intense P acquisition (Olsson *et al.*, 2014). In our experiment, vesicles (as lipid-rich storage structures) were infrequent so that the impact of P fertilization on their occurrence cannot be evaluated properly (data not shown). On the other hand, both mycorrhizal P uptake response and frequency of arbuscules was decreased by P-fertilization in our study, indicating lower symbiotic exchange activity of AMF. This again suggests the coupling of P and C symbiotic flows. However, decreased C-sink activity of AMF lipids might be caused by P-fertilization independently on plant actions. Whereas direct P-toxicity for AMF is unlikely in our P-treatments, the indirect effect of P mediated e.g. by shifts in abundance/composition of soil microbiota cannot be ruled out, although we did not see any indications for such explanation.

Another question is whether the reduced C flow to AMF, observed in our experiment in P-fertilized soil, was not caused indirectly by the generally reduced C flow to roots in the P-rich plants. The reduced plant C allocation to belowground due to fertilization could be anticipated at the basis of both previous observations and plant-economy theories (Bloom *et al.*, 1985; Lemoine *et al.*, 2013; Řezáčová *et al.*, 2017b). However, the ratio of C allocated to roots vs. shoots was not influenced by P fertilization in our study. In contrast, the fraction of root C share gained by AMF was lower due to P-fertilization, i.e. the decrease of C flow due to P-fertilization was specific to AMF. This still does not necessarily mean direct action of plant. For example, an experiment with *Sorghum* plants inoculated by *G. fasciculatus* revealed that reduced AMF colonization under high P might be caused by decreased permeability of root membranes, accompanied with decreased exudation of sugars and amino acids, and that it could be overcome by increased temperature (Graham *et al.*, 1982).

In conclusion, there is no unequivocal evidence that the decreased C income of AMF under P fertilization in our experiment was imposed directly by plants employing restriction of C flow, although the data fit well with this explanation.
8 Conclusions

The thesis highlights the importance of both light conditions and soil P fertility for functioning of arbuscular mycorrhiza. The main findings are:

- Symbiotic P flow from AMF to the host plant can be changed rapidly after the change of light condition. Specifically, P gained by AMF from soil may be either redirected to another host or retained in AMF mycelia if no suitable host is available. This opens the way for AMF to hold a bargain power despite being completely dependent on their host.
- Mycorrhizal plants can acclimatise to the pressing C sink of AMF under light-deprivation by changes in shoot morphology (besides others).
- Symbiotic C flow from plant to AMF can decrease in P-fertilized soil independently on the C flow to roots and on the current mass of AMF. This may indicate the active role of plant in regulation of C income of AMF.
- Experimentally based estimates of symbiotic C cost of AMF usually fell well below 10% of host plant photosynthetic production, while higher values were observed infrequently.

In conclusion, this thesis brings new pieces of knowledge about the symbiotic functioning of arbuscular mycorrhiza. There is increasing evidence of an active behaviour of both partners on symbiotic interface with the respect to current symbiotic supplies and demands. However, our knowledge of these aspects of such a widespread symbiosis is still highly limited. For example, there are striking knowledge gaps concerning the rate and sequence of changes in symbiotic functioning of arbuscular mycorrhiza after sudden light-shortage of plants which appeal for new research.
9 Závěry (in czech)

Tato práce poukazuje na význam světelných podmínek a hojnosti P v půdě pro fungování arbuskulární mykorrhizy. Hlavní poznatky jsou:

- Symbiotický tok P z arbuskulárně mykorrhizních (AM) hub do hostitelské rostliny se může rychle změnit kvůli změně světelných podmínek. Konkrétně, P získaný AM houby z půdy může být buď přesměrován do jiného hostitele, nebo zadržen v myceliu AM hub, pokud jiný vhodný hostitel není dostupný. To otvírá možnost, že AM houby disponují vyjednávací sílou, třebaže jsou zcela závislé na svých hostitelích.
- Mykorhizní rostliny se mohou přizpůsobit palčivým požadavkům AM hub na C v nepříznivých světelných podmínkách změnami v morfologii prýtu (mimo jiné).
- Symbiotický tok uhličit do hub může v půdě hnojené P klesnout nezávisle na toku C do kořenů a na aktuální hmotě AM hub. To možná ukazuje na aktivní úlohu rostliny při regulaci příjmu C houby.
- Experimentálně podložené odhady symbiotické uhličité ceny AM hub obvykle padají dosti pod 10 % fotosyntetické produkce rostlinných hostitelů, zatímco vyšší hodnoty byly pozorovány zřídka.

Závěrem, tato práce přináší nové poznatky o symbiotickém fungování arbuskulární mykorrhizy. Vzrůstá počet důkazů svědčících pro aktivní chování obou partnerů na symbiotickém rozhraní vzhledem k aktuálním symbiotickým dodávkám a požadavkům. Nicméně, naše znalosti o uvedených aspektech této tak velice rozšířené symbiózy jsou stále velmi omezené. Například máme propastný nedostatek poznatků o rychlosti a sledu změn v symbiotickém fungování arbuskulární mykorrhizy při nenadálém nedostatku světla pro rostliny, což volá po dalším výzkumu.
10 References


11 Acknowledgement

I thank from my heart everyone who supports me to finish this PhD thesis.

I gratefully acknowledge the tremendous work and support by my supervisor, Jan Jansa, who introduced me to the fascinating topic of arbuscular mycorrhiza and lead me through my work. I would like to thank all other members of our Laboratory of Fungal Biology at the Institute of Microbiology of Czech Academy of Sciences, who contributed highly to the fact that had a pleasure from my work. Special thanks belong to Veronika Řezáčová, who always support and encourage me. I am very grateful for the wonderful friendly atmosphere at the Department of Experimental Plant Biology of Faculty of Science, Charles University, and I thank Lukáš Fisher and Jana Albrechtová to encourage me to early submission of my thesis. Special thanks belong to Viktor Žárský and Jan Borovička, who trusted me to employ me.

I do appreciate the help of my family and my friend, Ivo Michal, who helps me substantially with the English (but all mistakes in the text are solely mine).

This work was supported by projects LK11224 and LO1417 granted by the Ministry of Education, Youth and Sports of the Czech Republic and by the long-term development program RVO61388971.
Appendix

Appendix is not publically available in the electronic version of the thesis, because it contains published works and works which are being published.

Appendix consist of following files:

12.1 Publication 1

12.2 Supplementary material for Publication 1
Both files are freely available on the website of Frontiers in Plant Science:
http://journal.frontiersin.org/article/10.3389/fpls.2015.00065/full

12.3 Additional figures for Publication 1
Photo-documentation of the experiment and the representative photos of the shoots for the morphological analysis.

12.4 Publication 2

12.5 Supplementary material for Publication 2
Both files are freely available on the website of Frontiers in Plant Science:

12.6 Publication 3
This book chapter should be available from the Springer Publisher due July 21, 2017.

12.7 Publication 4

12.8 Supplementary material for Publication 4
This manuscript is submitted to the Plant and Soil, but the final decision has not been made to the date of the thesis submission. Maybe someday the revised version of the files will be available on the website of the journal.

12.9 Abstract of an unpublished experiment

12.10 Statements of contribution
The statements are literally the same as in the section 6, but the signatures of the corresponding authors (or one other author and the supervisor, if the student is the corresponding author) are attached.