COMPETITION AND FACILITATION AMONG GRASSLAND PLANTS

- THE ROLE OF ARBUSCULAR MYCORRHIZA -

Dissertation

zur Erlangung des akademischen Grades

Doktor der Naturwissenschaften (Dr. rer. nat.)

-

Fakultät für Biologie

Universität Bielefeld

vorgelegt von

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Bielefeld, Dezember 2014

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ACKNOWLEDGEMENTS

Vielen Dank an

Prof. Wolfram Beyschlag, für die Ermöglichung dieser Arbeit am Lehrstuhl für Experimentelle Ökologie und Ökosystembiologie, und für die vielen hilfreichen wissenschaftlichen Diskussionen und Anregungen.

Dr. Stephan Unger, für die fortwährende und beinahe grenzenlose Hilfsbereitschaft bei der Konzeption und der praktischen Umsetzung der Experimente, der Interpretation der gewonnen Ergebnisse und ihrer Verschriftlichung, und nicht zuletzt für die herzliche Arbeitsatmosphäre.

Prof. Christiane Werner, für die vielen konstruktiven Diskussionen und Ratschläge zu den Experimenten und der Auswertung der gewonnen Ergebnisse.

Dr. Tom Steinlein, für ein stets offenes Ohr und viele nützliche Ratschläge zu Anliegen aller Art, nicht zuletzt für seine Hilfe bei Statistikfragen.

Prof. Caroline Müller, für die freundliche Erstellung des Zweitgutachtens.

Martina Friede, Margita Hefner und Verena Lauströer, für das Interesse an meinem Forschungsthema und das große Engagement in einem Teil der Experimente.

Elke Furlkröger, Barbara Teichner und Christine Schlüter, ohne die die praktischen Arbeiten in Klimakammer, Gewächshaus und Labor niemals hätten bewältigt werden können. Vielen Dank für Eure großartige Unterstützung und die unendlich vielen guten Gespräche!

Angelika Mühlbrandt, für die Hilfe bei administrativen Angelegenheiten und Bestellungen.

Dr. Frederik Wegener, für den großen Beitrag zum angenehmen Arbeitsklima am Lehrstuhl und zu einer Vielzahl netter Abende außerhalb des Lehrstuhls... und selbstverständlich für die vielen wertvollen Tipps rund um Theorie und Praxis von Gaswechsel- und Labelingversuchen.

Dr. Christian Platner, für die Hilfe bei Statistikfragen, die vielen Denkanstöße in wissenschaftlichen (und auch nicht-wissenschaftlichen...) Diskussionen und die nette Atmosphäre am Lehrstuhl.

Alex, Freddy, Juliane, Katie, Katrin, Maren, Martina und Tine, für den wissenschaftlichen Austausch, die gemeinsame Zeit und die lockere und freundschaftliche Atmosphäre am Lehrstuhl.

Dr. Rabea Schweiger, für den wissenschaftlichen Austausch auf theoretischer und materieller Ebene.

Dr. Helga Pankoke und Agniezska Matuszak, für die gute Zusammenarbeit und den wissenschaftlichen Austausch.

Dr. Marjan Jongen, für hilfreichen Input während der Erstellung des Manuskripts zu Exp. 1.

Dr. Maik Bartelheimer, für wertvolle Denkanstöße auf dem Gebiet der Wurzelkonkurrenz.

Nils Köhler, Thorsten Schäfers, Siegfried Berger, Paul Müller, Gerhard Drexler, Horst-Jürgen Nosseler und allen anderen Mitarbeitern der Mechanischen Verbundwerkstatt Biologie-CITEC, für stets schnelle und in vollstem Maße zufriedenstellende Herstellung anspruchsvoller Versuchsmaterialien.

Uschi Büchner, Marcus Höpfner, Detlev Balten und allen anderen Mitarbeitern der Gärtnerei, für die stetige Hilfsbereitschaft bei Anliegen aller Art, insbesondere für das Autoklavieren von - gefühlt mehreren Tonnen - Sand.

Prof. Matthias Rillig und Mitarbeitern, für die Einführung in die Grundlagen der Mykorrhiza-Analytik.

Marina Wolf, für die Ermöglichung des Ausglühens großer Mengen Sand in den Töpferöfen der Fakultät für Kunst- und Musikpädagogik.

Sarah Kindermann (wir werden dich immer in Erinnerung behalten), David Behringer, Helge Landskron und Alexandra Kassis, für die tatkräftige Unterstützung bei verschiedenen Versuchen.

Und noch einmal Alexander Mosena, für den Beitrag zur guten Stimmung im Lehrstuhl, ein stets offenes Ohr, die noch nicht ganz abgeschlossene "Frankonisierung" und zahlreiche legendäre Sessions.

Meiner Familie und meinen Freunden, für den fortwährenden Zuspruch und die vielfältige Unterstützung!

Lisa, die immer für mich da war.

INDEX OF ABBREVIATIONS

abbreviation	meaning
$\delta^{13}C$	carbon isotope composition (VPDB-Standard)
µm	micrometer
µmol	micromole
AM	arbuscular mycorrhizal (refers to plants inoculated with AMF)
AMF	arbuscular mycorrhizal fungi
ANCOVA	analysis of covariance
ANOVA	analysis of variance
atom% ^{13}C	percentage of ^{13}C atoms related to total C in a sample
C	carbon
$CaCl_2$	calcium chloride
$Ca(NO_3)_2$	calcium nitrate
Cc	*Corynephorus canescens*
cm	centimeter
cm^3	cubic centimeter
CMN	common mycelial network
CO_2	carbon dioxide
CRDS	cavity ring-down spectroscopy
CRDS-CFI	cavity ring-down spectroscopy continuous flow injection
$CuSO_4$	copper sulfate
d	days
dap	days after planting
dpi	dots per inch
ER	extraradical (i.e., outside plant roots)
ERM	extraradical hyphae
Exp.	experiment
$FeC_6H_5O_7$	iron citrate
FIA	flow injection analysis
Fp	*Festuca psammophila*
g	gram
h	hour

H_3BO_3	boric acid
Hp	*Hieracium pilosella*
Hr	*Hypochaeris radicata*
KCl	potassium chloride
kg	kilogram
KH_2PO_4	monopotassium phosphate
KNO_3	potassium nitrate
KOH	potassium hydroxide
l	liter
LP	large plant
LPC	large plant compartment
m^2	square meter
MGD	Mycorrhizal Growth Dependency
$MgSO_4$	magnesium sulfate
ml	milliliter
min	minute
mm	millimeter
mm^3	cubic millimeter
mmol	millimole
$MnSO_4$	manganese sulfate
MoO_3	molybdenum trioxide
n	number of replicates
n.s.	not significant
$(NH_4)_2HPO_4$	diammonium phosphate
$(NH_4)_2SO_4$	ammonium sulfate
NM	non-mycorrhizal (refers to plants not inoculated with AMF)
nm	nanometer
P	phosphorus
Pl	*Plantago lanceolata*
PPFD	photosynthetically active photon flux density
RLP	relative respiratory label-partition in LP-compartments
rpm	rounds per minute
r/s-ratio	root-to-shoot ratio
RCI	Relative Competitive Intensity

RNE	Relative Neighbor Effect
s	second
SA	surface area
SC	seedling compartment
SGH	stress gradient hypothesis
$ZnSO_4$	zinc sulfate

SUMMARY

Interactions between plants generally are of great importance for successional processes and plant community structure and thus, research on the underlying mechanisms is essential for a attaining a comprehensive understanding in the field of vegetation ecology. In this regard, the relevance of belowground interactions has long been neglected, although they represent the dominant interaction type in several biomes of global importance, such as grassland ecosystems. In particular, the impact of the mutualistic relationship between plants and arbuscular mycorrhizal fungi (AMF) has been ignored for a long time. Although it has been revealed that AMF are involved in the nutrition of the majority of grassland plants and may have considerable influence on belowground plant-plant interactions between them, there is still only poor knowledge on the underlying mechanisms. Intensive research is particularly required on the relevance of mycotrophy (i.e., the species-specific dependency of a plant on AMF for nutrient acquisition) for the competitive ability of a plant, as well as on the mediation of plant-plant interactions via common mycelial networks (CMNs; mycorrhizal mycelia that interlink different plant individuals). The present study addresses these questions and presents the results of four controlled pot experiments in this connection. The experiments were performed on five grassland plant species of Central Europe.

In a first experiment (Ch. 2), competition between the grassland forbs *Hieracium pilosella* and *Plantago lanceolata* was investigated to test the suitability of foraging via AMF compared to foraging via roots with respect to the competitive ability. The results revealed that, although a highly mycotrophic life-style (with predominantly AMF-mediated foraging) may be a very successful trait on the individual scale, it may be a disadvantageous trait for the competitive ability as compared to a more root-mediated nutrition. Further, it is concluded that for making predictions on the outcome of a competitive interaction, both, the mycotrophy level as well as root properties of the involved plants need to be considered.

The second experiment (Ch. 3) compared differences between growth dynamics and nutrient depletion capacities of mycorrhizal hyphae and roots between the coarse-rooted forbs *P. lanceolata*, *H. pilosella* and *Hypochaeris radicata*, and the fine-rooted grasses *Corynephorus canescens* and *Festuca psammophila*. The results demonstrated completely contrasting foraging strategies (i.e., AMF-mediated vs. root-mediated nutrient acquisition) in potentially competing plant species. It further revealed that in highly mycotrophic plants, initiation of an adequate phosphorus (P) uptake is strictly dependent on presence of AMF. Nevertheless, the results led to the conclusion that AMF-mediated foraging may provide some

(competitive) advantages over root-mediated foraging in terms of rapid exploitation of P from of bare soil patches, due to comparatively high growth rates of hyphal absorptive surface area.

CMN-effects on plant-plant interactions and the underlying mechanisms were investigated in two seedling facilitation experiments (Ch. 4 and 5), using pots with AMF-accessible, root-excluding compartments as main experimental tool to achieve a separation of CMN- from root-mediated interactions. The results showed that the main CMN-mediated facilitative adult plant effect on seedlings was an accelerated mycorrhizal colonization of seedling roots, which occurred to be particularly critical for P-uptake and seedling establishment of highly mycotrophic species. Promotion of CMN-growth should be highest in adult plants belonging to productive, highly mycotrophic species, such as *P. lanceolata*, which revealed as a potential 'key species' for CMN-growth. Nevertheless, high adult plant carbon-investment into a CMN did not reduce the CMN-costs to seedlings, but, in contrast, rather increased them. Further, the two facilitation experiments revealed that (root- and CMN-mediated) competitive pressure by adult plants may overlay any facilitative effects, resulting in net neutral or negative effects on seedling growth. Net CMN-mediated seedling facilitation might be highest when pronounced mycelium growth is combined with low competitive pressure by the adult plant, as e.g., exhibited by the highly mycotrophic forb *H. pilosella*.

Summarizing, this study demonstrates the generally high relevance of mycorrhizal parameters for plant-plant interactions and emphasizes the pronounced species-specificity of mycotrophy levels and CMN-effects. Regarding these factors, this study gives some new insights into the mechanisms underlying AMF-effects on plant-plant interactions.

ZUSAMMENFASSUNG

Interaktionen zwischen Pflanzen sind allgemein von großer Bedeutung für Sukzessionsprozesse und die Zusammensetzung von Pflanzengesellschaften, weshalb die Erforschung der zugrunde liegenden Mechanismen für ein umfassendes vegetationsökologisches Verständnis unabdingbar ist. Die Bedeutung unterirdischer Interaktionen wurde dabei lange unterschätzt, obgleich sie in einer Reihe global sehr bedeutsamer Biome, wie z.B. Graslandökosystemen, die vorherrschende Interaktionsform darstellen. Insbesondere der Einfluss der mutualistischen Beziehung zwischen Pflanzen und arbuskulären Mykorrhizapilzen (AMF) wurde dabei lange ignoriert. Obwohl sich gezeigt hat, dass AMF in die Nährstoffaufnahme des Großteils aller Graslandpflanzen involviert sind und einen beachtenswerten Einfluss auf unterirdische Interaktionen zwischen diesen haben können, ist über die zugrundeliegenden Mechanismen nur wenig bekannt. Intensive Forschungsarbeit ist hier insbesondere zur Bedeutung der Mykotrophie (d.h. des artspezifisch variierenden Grades, zu dem die Nährstoffaufnahme einer Pflanze von AMF abhängt) und der Bedeutung gemeinschaftlicher Mykorrhizanetzwerke (CMNs; d.h. Mykorrhizamyzelien, die verschiedene Pflanzenindividuen miteinander verbinden) erforderlich. Diese Themenbereiche sind Gegenstand der vorliegenden Dissertation, in der die Ergebnisse von vier in diesem Zusammenhang durchgeführten, kontrollierten Topfexperimenten vorgestellt werden. Die Versuche wurden an fünf Arten mitteleuropäischer Graslandpflanzen durchgeführt.

In einem ersten Experiment (Kap. 2) wurde die Konkurrenzbeziehung zwischen den Kräutern *Hieracium pilosella* und *Plantago lanceolata* untersucht, um die Bedeutung der AMF-vermittelten Nährstoffaufnahme für die Konkurrenzfähigkeit zu ermitteln, wobei diese mit der Nährstoffaufnahme über Wurzeln verglichen wurde. Wie die Ergebnisse zeigten, ist eine hoch-mykotrophe Lebensweise (mit überwiegend AMF-vermittelter Nährstoffaufnahme) zwar eine sehr erfolgreiche Strategie auf individueller Ebene, kann aber in Konkurrenz gegenüber einer eher wurzelvermittelten Nährstoffaufnahme einen Nachteil darstellen. Des weiteren konnten wir aus diesem Experiment schließen, dass sowohl die jeweiligen Mykotrophiegrade als auch die Wurzeleigenschaften der involvierten Pflanzen berücksichtigt werden müssen, um den Ausgang einer Konkurrenzbeziehung vorherzusagen.

Im zweiten Experiment (Kap. 3) wurden die Wachstumsdynamiken und Nährstoffabreicherungskapazitäten von Mykorrhizahyphen und Wurzeln zwischen den grobwurzeligen Kräutern *P. lanceolata*, *H. pilosella* und *Hypochaeris radicata* und den feinwurzeligen Gräsern *Corynephorus canescens* und *Festuca psammophila* verglichen. Die

Ergebnisse zeigten stark gegensätzliche - also AMF-vermittelte bzw. wurzelvermittelte Nährstoffaufnahmestrategien in potentiell konkurrierenden Pflanzenarten. Weiter zeigte sich, dass die Initiierung der Aufnahme von Phosphor (P) in hoch-mykotrophen Pflanzen strikt an die Präsenz von AMF gebunden ist. Nichtsdestotrotz lassen die Ergebnisse auf mögliche Vorteile AMF-vermittelter Nährstoffaufnahme gegenüber wurzelvermittelter Nährstoffaufnahme bezüglich der schnellen Ausbeutung von P aus freien (d.h. unbesiedelten) Bodenbereichen schließen, was auf die vergleichsweise schnelle Vergrößerung der absorbierenden Oberfläche von Hyphen zurückzuführen war.

CMN-Effekte auf pflanzliche Interaktionen und die zugrundeliegenden Mechanismen wurden in zwei Experimenten zur Keimlingsförderung (Kap. 4 und 5) untersucht. Das zentrale experimentelle Instrument zur Unterscheidung von CMN-vermittelten und wurzelvermittelten Interaktionen stellten hierbei Töpfe mit separaten Kompartimenten, die nur für AMF, nicht aber für Wurzeln zugänglich waren. Die Ergebnisse zeigten, dass der maßgebliche förderliche (CMN-vermittelte) Effekt adulter Pflanzen auf Keimlinge in einer beschleunigten Kolonisierung der Keimlingswurzeln durch AMF bestand, was insbesondere bei hoch-mykotrophen Pflanzenarten äußerst wichtig für P-Aufnahme und Keimlingsetablierung ist. Das Wachstum eines CMNs sollte insbesondere durch produktive, hoch-mykotrophe Arten wie *P. lanceolata* vorangetrieben werden, die sich hier als potentielle "key species" für das CMN-Wachstum offenbarte. Interessanterweise trugen hohe Kohlenstoffinvestitionen adulter Pflanzen in ein CMN allerdings nicht zur Reduktion der CMN-Kosten der Keimlinge bei, sondern hatten sogar eine Erhöhung dieser zur Folge. Weiter zeigten die beiden Förderungsexperimente deutlich, dass förderliche Effekte gänzlich von (wurzel- und CMN-vermittelten) Konkurrenzeffekten durch adulte Pflanzen überlagert werden können, so dass die Nettoeffekte schließlich neutral oder sogar negativ ausfallen. Die größten positiven Nettoeffekte sind zu erwarten, wenn eine adulte Pflanze ein starkes Myzelwachstum verursacht aber gleichzeitig nur einen geringen Konkurrenzdruck ausübt, so wie es beispielsweise bei dem hoch-mykotrophen Kraut *H. pilosella* der Fall war.

Zusammenfassend unterstreicht die vorliegende Arbeit die generell hohe Bedeutung von mykorrhizabezogenen Parametern für pflanzliche Interaktionen und zeigt deutlich die hohe Artspezifität von Mykotrophiegraden und CMN-Effekten. Hinsichtlich dieser Faktoren bietet diese Studie einige neue Einblicke in die Mechanismen, die der Beeinflussung pflanzlicher Interaktionen durch AMF zugrunde liegen.

CHAPTER 1
-
General Introduction

Grassland ecosystems cover about 20 percent of the earth's terrestrial surface (Jentsch & Beyschlag 2003), and play an important role in the global carbon (C) cycling and storage (Scurlock & Hall 1998). As these systems are an important part of the biosphere, understanding their ecology is fundamental, particularly with respect to problems associated with changing climatic conditions. However, even though there has been a lot of research on vegetation dynamics and plant community structures in grasslands, a comprehensive knowledge on the underlying mechanisms is still lacking, particularly in the field of plant-plant interactions. One explanation for this lack of knowledge is that especially questions concerning belowground processes have long been neglected due to a multitude of methodological barriers. This is particularly critical for progress in grassland ecology, as research of the last decades has revealed that the major proportion of interactions in grasslands occurs belowground. This thesis attempts to contribute to an improved understanding of the mechanisms underlying belowground interactions between grassland plants.

1.1 Strategies of nutrient acquisition in grassland plants

Plants require several essential resources, including carbon, oxygen, water and several essential macro- and micronutrients (Marschner 1997). According to Liebig's law of the minimum, each of these resources may limit plant growth, depending on its relative availability. Consequently, a plant spends its energy, i.e., photosynthetic carbohydrates, predominantly on those structures that enhance the acquisition of the respective limiting resource (Iwasa & Roughgarden 1984; Wilson 1988). Thus, a pronounced belowground-directed allocation of C is most frequently found in ecosystems where light is available in sufficient amounts and plant growth is more limited by soil nutrients - a situation which is a characteristic of grassland systems. However, in addition to a generally high root-directed C allocation, grassland plants evolved a variety of strategies for an efficient usage of belowground-allocated assimilates. Disentangling the specific functioning of these strategies both on the individual level and also with respect to plant-plant interactions, is urgently required for a better understanding of vegetation dynamics in grassland systems.

1.1.1 Root 'autonomous' strategies of nutrient acquisition

Under conditions of low nutrient availability, relatively few nutrients are located within the spatially limited depletion zone of a plant. Thus, mechanisms for an extension of the depletion zone are favorable adaptations to low nutrient availability. One of the most successful strategies of belowground C-investment seems to be the increase of root biomass. So plant species adapted to nutrient-poor soils often show remarkably high root-to-shoot ratios (Wilson 1988). Furthermore, root-morphological strategies like formation and maintenance of high proportions of fine roots and root hairs as a method of surface area enlargement, have the potential to substantially enlarge the depletion zone and thus improve the nutrition of a plant (Ryser & Lambers 1995; Lambers *et al.* 2008; Richardson *et al.* 2009). This strategy is successfully employed by a quantity of plant species, many of which are grassland species, particularly by members of the Pooideae, where high fine root proportions frequently are combined with an extensive root architecture, thus forming a dense root web, covering large soil volumes (Kutschera & Lichtenegger 1982). Moreover, several plant species exhibit physiological adaptations to low nutrient availability, such as releasing high amounts of root exudates to mobilize soil nutrients by means of ionic exchange (Dakora & Phillips 2002).

1.1.2 Nutrient acquisition strategies involving mutualists

Besides the above-mentioned 'autonomous' root strategies, which are referred to as 'root-mediated' in the following, there is a quantity of mutualistic relationships between plants and soil microorganisms. The basic principle in most of these associations is the transfer of assimilated C to soil microbes, which in return enhance the plant's nutrient uptake, such as e.g., the exudation of sugars to stimulate growth and activity of nutrient-mobilizing or N-fixing bacteria (for an overview see Dakora & Phillips 2002). Another very important strategy is the symbiosis with arbuscular mycorrhizal fungi (AMF), which are associated with 80 percent of all land plants (Smith & Read 2008), and are the dominant type of mycorrhiza in grasslands. Today there is plenty of evidence, that AMF represent a highly relevant factor for plant nutrition (Marschner & Dell 1994) and a quantity of ecological processes as e.g., resistence to drought (Augé 2001), enhanced herbivore defense (Gehring & Whitham 1994; Sikes *et al.* 2009) and soil aggregation (Rillig & Mummey 2006).

1.1.2.1 AMF-mediated nutrient acquisition

The basic element of mycorrhizal mutualism is a reciprocal exchange of nutrients, with transfer of plant-C to the fungal partner and transfer of soil nutrients from fungus to plant (Redecker *et al.* 2000). AMF are obligate biotrophs, i.e., they are completely dependent on plant-C for growth and reproduction (Parniske 2008). The AMF mycelium is partitioned into an extraradical mycelium in the soil and an intraradical mycelium inside the plant roots,

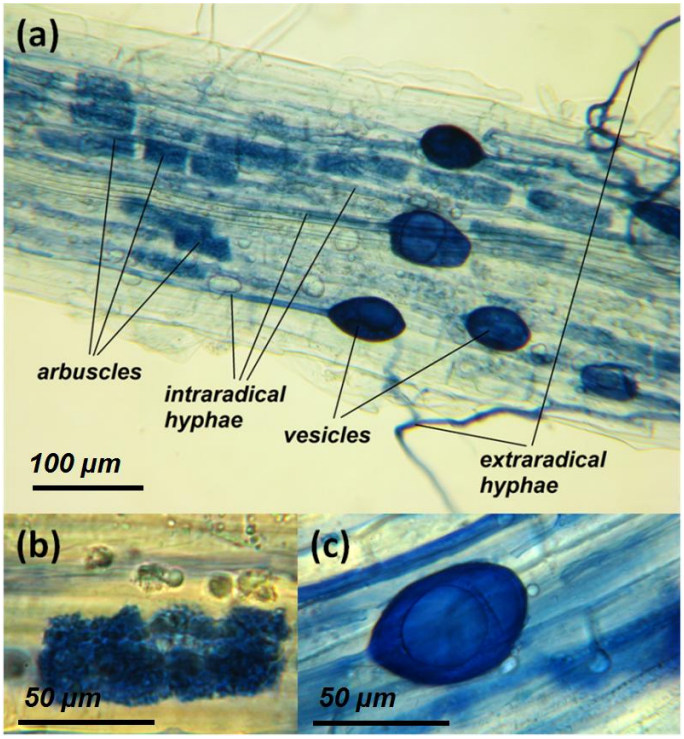

Fig. 1.1 Stained root of *Hieracium pilosella*, colonized by *Rhizophagus intraradices* (a) with arbuscules (b) and vesicles (c).

functioning in the acquisition of essential nutrients from the soil and in nutrient exchange with the plant, respectively (Parniske 2008; see also Fig. 1.1). In the following, 'AMF-mediated' nutrient acquisition refers to that proportion of acquired nutrients that was primary acquired via hyphae - irrespective of secondary transport through roots. Similar as in fine roots and root hairs, the biomass-to-surface ratio of mycorrhizal hyphae is very favorable for an efficient nutrient acquisition. Moreover, similar to extensive fine root systems, mycorrhizal hyphae may form dense mycelia in the soil, thus creating large depletion zones for an efficient exploitation of large soil volumes. Indeed, mycorrhiza may be seen as a functional equivalent of fine roots and root hairs (Baylis 1975; Brundrett 2002). It is known that AMF were already present in the earliest land plants, which had little-branched root systems and were almost certainly obligately dependent on their fungal partner for the acquisition of immobile phosphate ions (Pirozynski & Malloch 1975; Remy *et al.* 1994). The degree to which a plant

is dependent on AMF for its nutrition is usually described by the term 'mycotrophy' (Janos 2007). However, with ongoing evolution of complex root morphological and physiological nutrient acquisition strategies (see section 1.1.1), several plant species became less mycotrophic. Thus, within recent land plants, there is a broad spectrum of mycotrophy levels,

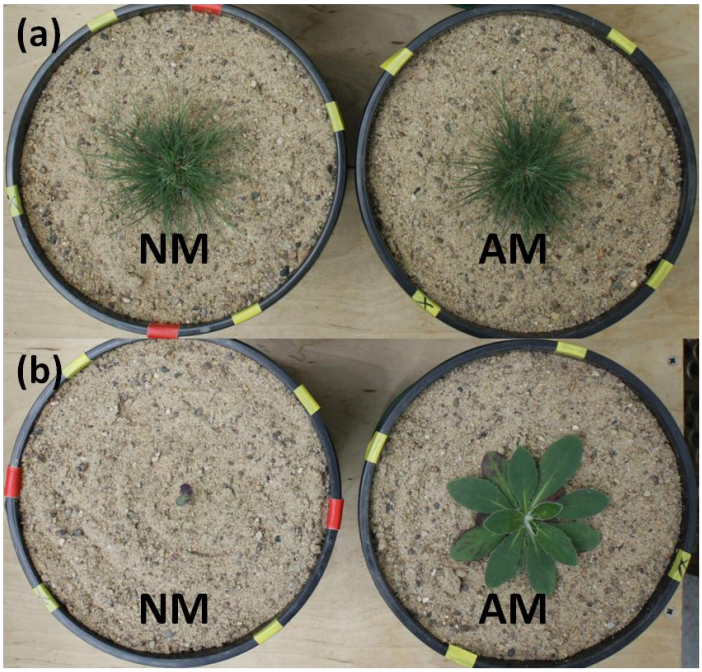

Fig. 1.2 Mycorrhizal (AM) and non-mycorrhizal (NM) plants of the weakly mycotrophic *Corynephorus canescens* (a) and the obligately mycotrophic *Hieracium pilosella* (b).

ranging from completely non-mycotrophic to facultatively or obligately mycotrophic plant species (Smith & Read 2008; see also Fig. 1.2). To make a complex phenomenon even more complex, the relevance of AMF for nutrient acquisition depends on a variety of abiotic and biotic factors, such as the availability of light, water and nutrients (Johnson 2010), as well as on interactions with other plants (e.g., Koide 1991; Facelli *et al.* 1999; Schroeder-Moreno & Janos 2008). Thus, although there has been a lot of research on mycorrhiza-mediated nutrient acquisition (for an overview see Clark & Zeto 2000), there is still a large number of open questions, especially regarding the interplay between AMF-mediated and root-mediated nutrient acquisition and the relevance of mycorrhiza for plant-plant interactions.

1.2 Implications of root- and AMF-mediated nutrient acquisition for competitive interactions between grassland plants

Understanding the mechanisms underlying plant-plant interactions is one of the main goals in ecological research, as interactions between plants are ubiquitous and in most cases important

drivers of ecosystem functioning. In this regard, particular attention is given to resource (exploitative) competition as the dominant interaction type in the majority of ecosystems, often playing an important role in structuring plant community composition (Tilman 1982; 1985; Grime 2001). In this context, Tilman (1982) defined resources as those environmental factors, that are altered in their availability due to the activity of organisms. Based on this definition, competition may be defined as 'an interaction between individuals, brought about by a shared requirement for a resource, and leading to a reduction in the survivorship, growth and/or reproduction of at least some of the competing individuals concerned' (Begon *et al.* 1998). In contrast to light-limited ecosystems with pronounced aboveground competition (e.g., forests), nutrient-limited systems, such as grasslands, are generally characterized by belowground competition (Casper & Jackson 1997; Weiner *et al.* 1997). Thus, strategies of efficient nutrient acquisition (see section 1.1) are not only adaptations to low nutrient availability but have frequently also important functions in competition for nutrients (Casper & Jackson 1997).

1.2.1 The role of roots in competition for nutrients

According to the definition of Goldberg (1990), competition for soil nutrients occurs when a plant has a negative effect on the availability of at least one nutrient to which another plant shows a positive response in growth, survival, or reproduction. The depletion of nutrients is thus a central element of plant competition, and, as mentioned above, may be achieved by several different root traits, regarding surface area and rates of resource uptake, morphological and physiological plasticity as well as spatial and temporal soil partitioning (Casper & Jackson 1997). However, one of the most efficient competition mechanisms is the creation of a large depletion zone by production of high root biomass and formation of highly branched and extensive fine root systems (Casper & Jackson 1997; Weiner *et al.* 1997; Hodge *et al.* 1999; Cahill & Casper 2000; Fitter *et al.* 2002), which can e.g., be found in a quantity of grassland species (Kutschera & Lichtenegger 1982; 1992a; b). Nevertheless, direct correspondence between root density and competitive ability is often lacking (e.g., Caldwell *et al.* 1991a; b), rising the question for other factors. In many cases, these discrepancies may - at least partially - be explained by associations with mycorrhizal fungi (see section 1.2.2), which participate in the nutrient acquisition of the great majority of land plants (Casper & Jackson 1997; Smith & Read 2008).

1.2.2 The role of mycorrhiza in competition for nutrients

The influence of mycorrhizal associations on competitive interactions has long been neglected, not least because of their 'invisibility' and several methodological difficulties in mycorrhizal analyses. However, during the last three decades, there is an increasing research interest in the role of mycorrhiza in competition (e.g., Fitter 1977; Grime *et al.* 1987; Marler *et al.* 1999; Scheublin *et al.* 2007). Today, it is widely accepted that mycorrhiza may be an important variable in competitive plant-plant interactions (Casper & Jackson 1997) and that it is highly relevant for vegetation dynamics and species composition of plant communities. This is particularly relevant for grassland systems, as these are hotspots of AMF abundance (Wang & Qiu 2006). AMF have been shown to amplify (e.g., Hetrick *et al.* 1994; Moora & Zobel 1996; Scheublin *et al.* 2007) competition or to shift dominance from one competitor to another (e.g., Grime *et al.* 1987; Hartnett *et al.* 1993; Daisog *et al.* 2012). However, the mechanisms by which AMF may influence competition revealed as quite diverse and complex, and research on the underlying mechanisms is still in its infancy. One reason for the high complexity is that mycorrhizal benefits gained by the competitors are dependent on a multitude of interacting factors, such as species identities of involved plants and fungi, nutrient and water availability, light availability as well as chemical and physical soil properties (see Hoeksema *et al.* 2010 for an overview). As revealed by the recently available literature on this topic, a complete understanding of AMF-related competition mechanisms requires intensive research, particularly in the fields of common mycelial networks (CMNs; e.g., van der Heijden & Horton 2009; Merrild *et al.* 2013) and mycotrophic degrees of competing plants (e.g., Janos 2007; Hoeksema *et al.* 2010; Johnson 2010). These topics will be focus of the present thesis.

1.2.2.1 AMF-mediated vs. root-mediated nutrient depletion: relevance of mycotrophy levels for the competitive ability of a plant

It is widely accepted that nutrient depletion via extensive and highly branched root systems is an appropriate and effective trait in competitive interactions (e.g., Casper & Jackson 1997; Weiner *et al.* 1997; Fitter *et al.* 2002). However, suggesting that the capacity to deplete nutrients is the key factor for the competitive ability of a species (see section 1.2.1), the outcome of competition should not depend on whether depletion is due to root- or AMF-mediated foraging, but should rather depend on the total absorptive surface. As belowground competition between neighboring root systems is size-symmetric (Weiner 1986; Weiner *et al.* 1997; Cahill & Casper 2000; Bartelheimer *et al.* 2008), the outcome of a competitive relation-

ship may be predicted based on the relation between the size of the depletion zones of the involved root systems. Nevertheless, in several cases size-symmetry could not be proved (e.g., Fransen *et al.* 2001; Facelli & Facelli 2002; Rajaniemi 2003) and one likely reason for this is that certain proportions of nutrients are depleted via AMF (Schwinning & Weiner 1996), dependent on the species-specific mycotrophy levels. Although it has been revealed that the mycotrophy levels of plants might play an important role in competition (e.g., Grime *et al.* 1987; Hetrick *et al.* 1994; Scheublin *et al.* 2007), there is still only poor knowledge about the implications of a highly mycotrophic life-style for the competitive ability of a plant. In particular, it is still unclear, if the process of AMF-mediated nutrient depletion (analogous to root-mediated depletion) may function as a competitive mechanism. This is an important question, since in highly mycotrophic plants, the major proportion of nutrients is acquired via the AMF-mycelium (Janos 2007). Moreover, potential competitive benefits as a consequence of a highly mycotrophic life-style have to be investigated with respect to nutrient availability and the spatial and temporal growth characteristics of roots and AMF-mycelia. Finally, representing one of the most important differences between direct root competition and AMF-mediated competition, the emergence of CMNs, interlinking competing plants (see section 1.2.2.2), requires special attention.

1.2.2.2 Plant-plant interactions via common mycelial networks

CMNs may interlink plant individuals of different species, age and size, and were found in all plant communities tested for their presence (Leake *et al.* 2004; van der Heijden & Horton 2009), indicating a potentially high ecological relevance of these networks. Such a connection between neighboring (mycorrhizal) plants may have important implications for their competitive relationship. In contrast to root-mediated nutrient acquisition, where the absorbed nutrients are exclusively available to a plant, both in absence and presence of a competitor (Fig. 1.3a, b), exclusive access to nutrients absorbed by an extraradical AMF mycelium is only prevalent in absence of (mycorrhizal) competitors (Fig. 1.3c). In competition, two (or more) plants are connected to the same AMF-mycelium and may compete for nutrients acquired by this CMN (Newman *et al.* 1992; Fig. 1.3d). Although recent investigations point towards allocation of plant-C to a CMN probably being one of the key factors for the distribution of CMN-nutrients between competitors (Merrild *et al.* 2013; Fellbaum *et al.* 2014), further research is required to achieve a comprehensive understanding of the underlying mechanisms. In particular, nothing is known about the implications of the species-specific

mycotrophy levels of the competitors connected via CMN, even though this trait is directly related to the proportions of both, C allocated to AMF, and nutrients acquired from the AMF.

As another important difference to root-mediated interactions, CMN-mediated interactions may also be positive, i.e., the performance of one plant can be facilitated by another (Leake *et al.* 2004; van der Heijden & Horton 2009). This makes the quantification of CMN-mediated competition even more difficult, as it has to be considered that the observed net interaction effect represents the sum of positive and negative interactions. Even though CMN-

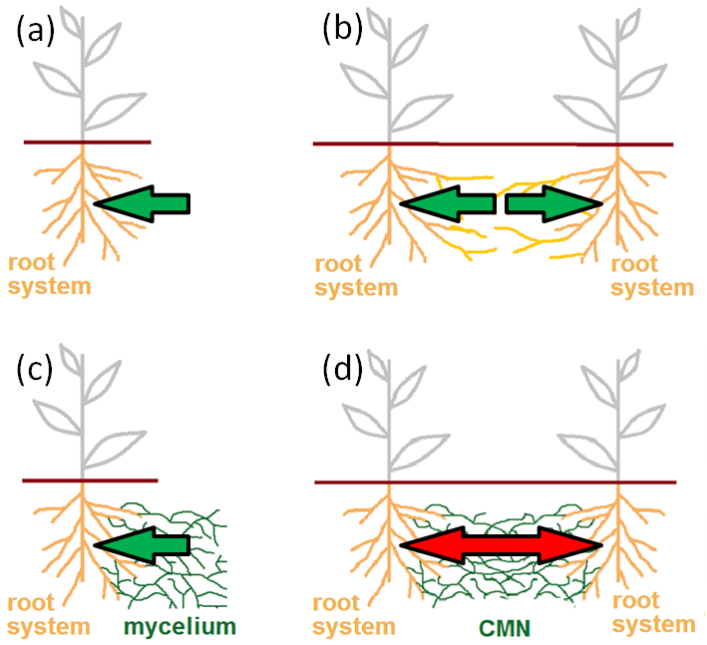

Fig. 1.3 Schematic depiction of the principal difference between root- (a, b) and AMF-mediated (c, d) nutrient acquisition. AMF-acquired nutrients are only exclusively available (green errors) to a plant in a monoxenic system, while they have to be shared (red errors) with other mycorrhizal plants due to emergence of a CMN.

mediated facilitation, particularly that of seedlings, has been observed several times (e.g., Grime *et al.* 1987; Friese & Allen 1991; Francis & Read 1995; van der Heijden 2004), the underlying mechanisms are still poorly understood. In this regard, one of the most important questions about CMN-mediated facilitation is, if there may be certain plant species, maintaining a CMN by high C-contributions (van der Heijden & Horton 2009). As C-allocation to AMF is supposed to be positively correlated with the mycotrophy level of a plant, highly mycotrophic plants might be potential 'key species' for construction and maintenance of AMF-mycelia. However, it is unclear if accelerating the process of mycorrhizal root colonization (Leake *et al.* 2004) is the only mechanism of seedling facilitation or if there might be further CMN-mediated advantages to seedlings, such as e.g., lower CMN maintenance costs to seedlings as a result of high adult plant C-investments. Finally, for

understanding net AMF-effects on plant competition, the interplay between the mechanisms underlying positive and negative CMN-mediated interactions has to be disentangled.

1.3 Concept of investigation

The main objective of this thesis was to disentangle mechanisms underlying root- and AMF-mediated foraging and unraveling the implications of their interplay for the competitive ability of a plant. Controlled pot experiments were performed using highly and lowly mycotrophic grassland plant species which occur in nutrient deficient habitats. To work out this main objective, three subordinate objectives were investigated in four experiments:

Objective 1) Disentangling the implications of a strongly mycotrophic life-style for the competitive performance of a plant.

- Exp. 1: The competitive relationship between a highly mycotrophic plant with a small root system and a less mycotrophic plant with a large root system was compared between presence and absence of AMF to unravel potential competitive advantages by AMF-mediated foraging, distinguishing between different levels of nutrient availability. (Ch. 2)

Objective 2) Disentangling potential advantages of AMF-mediated over root-mediated foraging in terms of proliferation into uncolonized soil patches and phosphorus (P) depletion from these.

- Exp. 2: Facultatively mycorrhizal grasses and obligately mycorrhizal forbs were compared with respect to the growth rates of the total belowground absorptive surface area, distinguishing between the contributions of root and hyphal growth and their relation to the depletion of soil P. (Ch. 3)

Objective 3) Disentangling the role of CMN-mediated facilitation in interactions between plants of different mycotrophy level and unraveling the underlying mechanisms.

- Exp. 3: Intra- and interspecific facilitation of seedlings by adult plants via CMN and its relative importance for the net outcome of the interaction was compared between a highly mycotrophic plant species with a small root system and a less mycotrophic plant species with a large root system. (Ch. 4)
- Exp. 4: In a novel microcosm approach combined with ^{13}C-labeling, growth, nutrition and CMN-C-costs of seedlings were analyzed as dependent on the species-specific

contributions to CMN-establishment and -maintenance by adult plants of distinct mycotrophy levels. (Ch. 5)

CHAPTER 2

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Experiment 1

Obligate mycotrophy: Can a strongly mycorrhiza-mediated foraging strategy serve as an effective mechanism in interspecific competition?

2.1 Abstract

AMF form extensive mycelia, considered to serve as a substitute for root surface. Highly mycotrophic plants often have relatively smaller root systems than less mycotrophic species, indicating a trade-off between the different foraging strategies. To test the suitability of both strategies in interspecific competition we investigated the impact of mycorrhization on competitive interactions between the herbs *Hieracium pilosella* (obligatory mycotrophic) and *Plantago lanceolata* (less mycotrophic). Both species were grown with and without AMF in a controlled experiment, using two competition and three different fertilization treatments to induce diverse mycorrhizal growth responses. Species-specific differences in mycotrophy were reflected in both root/shoot allocation and mycorrhizal responsiveness. In contrast to *P. lanceolata, H. pilosella* exhibited higher investment in shoot biomass (and clonal growth) with increased nutrient availability. *P. lanceolata* dominated competition at all fertilizer levels, enabled by its comparatively large root system and an unexpected high mycorrhizal responsiveness. Competitive strength of *P. lanceolata* was amplified by the presence of AMF. Unrelated to AMF, the competitive imbalance was reduced in favor of *H. pilosella* under nutrient deficiency. The poor competitive performance of *H. pilosella* indicates that AMF-mediated foraging may be less effective than root-mediated foraging in competitive interactions. However, high mycorrhizal benefits of *H. pilosella* might enable rapid establishment of closed aggregations, thereby reducing interspecific competition at the community level. Our results emphasize the importance of root parameters and nutrient availability for interpreting the outcome of interspecific competition between species of different degrees of mycotrophy.

2.2 Introduction

Competitive interactions between plants are influenced by a variety of abiotic and biotic factors, such as nutrient availability, climate, pathogens, herbivores and other parasitic or

mutualistic biotic interactions (e.g., Grime *et al.* 1987; Tilman 1988; Aerts 1999; Brooker 2006). AMF, which form close and typically mutualistic associations with herbaceous plant species and which may establish CMNs interlinking competing host plants, are of particular importance in this context (Grime *et al.* 1987; van der Heijden *et al.* 1998, 2003; Carey *et al.* 2004; Scheublin *et al.* 2007; Facelli *et al.* 2010). Although various studies demonstrated that AMF can considerably affect the competitive relationships in plant communities and in successional processes (Hart *et al.* 2003; Janouskova *et al.* 2011), the underlying mechanisms are still poorly understood and require further investigation.

There is a broad spectrum of potential mechanisms by which AMF may affect the competitive ability of their host plants. For instance, AMF are able to enhance the host plant's resistance against different forms of stresses, such as drought, soil pathogens or herbivores (Gange & West 1994; Augé 2001; Sikes *et al.* 2009). However, the most important factor and fundamental element of the plant-fungal association is the exchange of matter between the two partners, in particular the translocation of carbohydrates from the plant to the fungus, and vice versa the transfer of soil nutrients (primarily P) from the AMF to the plant (Parniske 2008; Smith & Read 2008). Most interestingly, the ratio between carbon costs and nutritional benefits for the plant can range from cases of clear mutualism to fungal parasitism (Koide 1985; Modjo & Hendrix 1986; Bougher *et al.* 1990; Fitter 1991; Smith & Smith 1996; Johnson *et al.* 1997; Johnson 2010) resulting in a large variety of mycorrhiza related growth responses of the host (e.g., Janos 2007 and references therein). Direction (positive or negative) and extent of the responsiveness (i.e. the growth difference between inoculated and non-inoculated plants; Janos 2007) depend on factors like biotic and abiotic soil properties, light intensity and species-specific traits of plant and fungal partners (Hayman 1974; Gerdemann 1975; Johnson *et al.* 1997; Klironomos 2003; Jones & Smith 2004; Smith & Read 2008). A recently published meta-analysis (Hoeksema *et al.* 2010) identified ‘plant taxonomy’ and ‘plant functional group’ as the two most important traits affecting the dependency of a plant on fungus related nutrition (mycotrophy; e.g., Janos 2007). Other studies identified photosynthetic capacity as an important factor explaining diverse degrees of mycotrophy. For example, C_4-grasses exhibited higher positive mycorrhizal growth responses than C_3-grasses and their higher photosynthetic capacity appeared to directly affect carbon allocation to the AM symbionts (Wilson & Hartnett 1998; Hartnett & Wilson 1999; Hoeksema *et al.* 2010).

Further, mycotrophy has often been linked to root architecture, typically exhibiting a negative correlation between root specific surface area and mycorrhizal responsiveness, interpreted as a result of ~400 million years of co-evolution between fungi and plants (Baylis

1975; Brundrett & Kendrick 1988; Hetrick *et al.* 1992; Fitter & Moyersoen 1996; Brundrett 2002; Seifert *et al.* 2009). Obviously mycorrhizal fungi can act as a substitute for root surface area and highly mycotrophic plant taxa often decrease biomass allocation to roots, as compared to less mycotrophic taxa (Azcón & Ocampo 1981; Johnson 2010). The negative correlation between root biomass and the degree of mycotrophy reflects a trade-off between two strategies of carbon investment both aiming to enlarge the belowground absorbing surface in order to expand the soil nutrient depletion zone.

The capacity to enhance the efficiency of nutrient acquisition and depletion from soils by increasing either root or mycorrhizal absorption surface area is not only important on the individual scale but may be a crucial determinant of belowground competitive success (Eissenstat & Volder 2005; Weigelt *et al.* 2007). While it is widely accepted that nutrient depletion via extensive and highly branched root systems is an appropriate and effective trait in competitive interactions (Casper & Jackson 1997; Weiner *et al.* 1997; Hodge *et al.* 1999; Cahill & Casper 2000; Fitter *et al.* 2002), this strategy seems unlikely in highly mycotrophic species as they are thought to deplete nutrients mainly via AMF instead of the root system (Brundrett 2002). This raises the question whether this is a similarly efficient competition strategy as the formation of large root systems. The majority of the available literature supports the relevance of a high degree of mycotrophy for enhanced competitive strength (Allen & Allen 1984; Grime *et al.* 1987; Hetrick *et al.* 1989; Hartnett *et al.* 1993; Hetrick *et al.* 1994; Crush 1995; Smith *et al.* 1999; Scheublin *et al.* 2007), but there are also contrasting studies (Hodge 2003; Daisog *et al.* 2012).

Relating mycorrhizal effects on competition to potential C-allocation strategies from the available literature is challenging, as belowground carbon allocation has rarely been quantified. Furthermore, the majority of the studies does not consider nutrient availability, which may have a major influence because nutrient uptake efficiencies of roots and mycorrhizal hyphae have been shown to differ substantially (Jakobsen *et al.* 2005; Lambers *et al.* 2008). Therefore, the success of both nutrition acquisition strategies might finally depend on nutrient availability and thus result in a differential outcome of the competitive relationship. Thus, both root biomass allocation and nutrient availability have to be taken into account to understand the relevance of mycotrophy for belowground competitive plant-plant interactions.

We performed a controlled pot experiment with *Hieracium pilosella*, an early successional pioneer plant in nutrient poor sandy soils described as an obligatory mycotrophic species with a relatively small root system (Kutschera & Lichtenegger 1992; Bishop & Davy

1994; van der Heijden *et al.* 1998) and *Plantago lanceolata*, a less mycotrophic grassland species with a comparatively large root system (Kutschera & Lichtenegger 1992; Gange & West 1994; Parádi *et al.* 2003; Ayres *et al.* 2006; Scheublin *et al.* 2007). We compared mycorrhizal growth responses of competing and single individuals of both species and applied three different levels of fertilization to induce different degrees of responsiveness, thus providing the possibility to evaluate mycorrhiza-mediated competition effects under conditions of differential mycorrhizal benefits for both species. We hypothesized (1) that in absence of AMF *P. lanceolata* would be the dominant species, (2) that presence of AMF would alter the competitive relationship in favor of *H. pilosella* and (3) that in presence of AMF *H. pilosella* would exhibit the highest competitive strength at the lowest fertilization level as AMF-mediated nutrition is expected to be most efficient under low nutrient availability.

2.3 Materials and methods

2.3.1 Experimental design

A controlled growth chamber experiment with *Hieracium pilosella* L. and *Plantago lanceolata* L. (Blauetikett-Bornträger GmbH, Offstein, Germany) was carried out in a randomized complete block design at the University of Bielefeld, Germany. We used three competition treatments (single grown *H. pilosella* individuals; single grown *P. lanceolata* individuals; *H. pilosella* and *P. lanceolata* individuals in competition), two mycorrhiza treatments (mycorrhiza present (AM); mycorrhiza absent (NM)) and three fertilization treatments (different fertilizer concentrations, termed ‘0.25’; ‘0.5’; ‘1’), with six replicates per treatment resulting in a total of 108 pots.

Seeds were sown and started in boxes with sterilized (120°C for 1.5 h) sand and grown during 14 days after germination. Subsequently seedlings were transplanted into pots of 2.700 cm^3 volume filled with sterilized sand. In the competition treatments plants were positioned symmetrically at a distance of 7 cm to each other and 4 cm to the pot edge. The single plants were also positioned at a distance of 4 cm to the pot edge to rule out treatment differences through edge effects. While transplanting the seedlings, 18 ml of a 1:1 mixture of sterilized sand and expanded clay inoculum, containing at least 200,000 infective units of *Rhizophagus intraradices* Schüssler & Walker per liter (BioMyc™ Environment GmbH, Brandenburg, Germany) was applied to the roots of each individual in the AM treatment. The NM seedlings received the same volume of a sterilized (120°C for 30 min) sand-expanded clay mixture.

Before sterilization, a microbial wash was extracted from the inoculum by sieving a solution of water and inoculum through a 20 µm sieve. 5 ml of the microbial wash were applied to each NM seedling in order to create a comparable soil bacterial community as compared to the AM treatment (Koide & Li 1989).

2.3.2 Growth conditions and plant nutrition

Plants were grown at a light / dark period of 14 h / 10 h with a temperature of 22 / 15 °C and relative air humidity of 60 %. PPFD was approx. 250 µmol m^{-2} s^{-1}. Since there was large variation in plant growth and thus in evapotranspiration, each pot was weighed once a week in order to individually calculate the water demand. Pots were watered twice a week with deionized water according to these specific demands to maintain soil moisture content between 6 and 8 % of the sand dry weight. Once a week a modified Hoagland fertilizer solution (Hoagland & Arnon 1950) was added to the water in concentrations matching the respective fertilization treatments: Based on the full concentration in fertilization level 1 (3 mmol KNO_3, 1 mmol $Ca(NO_3)_2$, 0.5 mmol $(NH_4)_2SO_4$, 0.5 mmol $(NH_4)_2HPO_4$, 1 mmol $MgSO_4$, 0.5 mmol KCl, 0.5 mmol $FeC_6H_5O_7$, 0.0125 µmol H_3BO_3, 0.001 µmol $MnSO_4$, 0.001 µmol $ZnSO_4$, 0.00025 µmol $CuSO_4$, 0.00025 µmol MoO_3; per liter) the solution was diluted to 50% and 25% for the fertilization levels 0.5 and 0.25, respectively. Fertilizer solution was always applied homogeneously across each pot to ensure equal distribution of nutrients between the competing plant individuals.

2.3.3 Harvest and data processing

After a growth period of 13 weeks all plants were harvested, and divided into root and shoot biomass (for *H. pilosella*, vegetative stolons were separated from leaf material). Roots of competing plants were carefully separated. In some cases small fractions of torn roots that could not be assigned to any of the competitors, was proportionally added to the root biomass of both species. All harvested plant material was dried at 70°C for 3 days and weighed. Total dry weights and root/shoot (r/s) - ratios were determined. Stolon dry weight in *H. pilosella* was added to shoot biomass.

For quantification of competitive effects, the Relative Neighbor Effect (RNE, Eq. 2.1), which is a modification of the Relative Competitive Intensity (RCI; Wilson & Keddy 1986) was calculated.

$$\text{RNE} = \frac{P_{\text{control}} - P_{\text{mix}}}{\text{x}} \quad \text{with}$$
$$\text{x} = P_{\text{control}} \text{ if } P_{\text{control}} > P_{\text{mix}}; \qquad \text{Eq. 2.1}$$
$$\text{x} = P_{\text{mix}} \text{ if } P_{\text{mix}} > P_{\text{control}}$$

where P_{mix} is the performance of a competing plant and P_{control} is the average performance of the corresponding single plants. Here, total dry weight was used as performance parameter. In contrast to RCI, RNE allows an equally rated assessment of competitive and facilitative interactions as the values vary between -1 and +1 with positive and negative values indicating competitive and facilitative effects, respectively (Markham & Chanway 1996).

For estimation of the species-specific degree of mycotrophy, Mycorrhizal Growth Dependency (MGD, Eq. 2.2) was calculated according to Smith *et al.* (2003):

$$\text{MGD} = 100 \times \frac{AM - NM}{AM} \qquad \text{Eq. 2.2}$$

where AM is the total dry weight of a mycorrhizal plant and NM is the average dry weight of the corresponding non-mycorrhizal plants. This index is based on the equation of Plenchette *et al.* (1983), resulting in values ranging from -∞ to +100 %, but was further adapted according to of Gange & Ayres (1999), allowing for calculation of variance as MGD values can be quantified for individuals. Although Smith *et al.* (2003) used the designation 'dependency', we used the MGD as a measure for the 'responsiveness' of plants to mycorrhization in terms of growth (see Janos (2007) for detailed discussion).

During the harvest, subsamples of fresh root material were taken for quantification of mycorrhizal colonization. These root fragments were cleared with 10 % KOH and treated with a 10 % ink-acetic-acid solution at 90 °C in order to stain the intraradical mycorrhizal structures (Phillips & Hayman 1970). The percentage of root length colonized by AMF was estimated using a modified intersection method (McGonigle *et al.* 1990), scoring a minimum of 100 intersections per sample for the presence of AMF.

2.3.4 Statistical analyses

Statistical analyses were performed using Statistica 6.0 (StatSoft Inc., Tulsa, USA). Significance level was set to $p < 0.05$. Data were tested for normal distribution by Kruskal-Wallis one-way analyses of variance. Data on biomass were analyzed for effects of competition and fertilization level using a two-way ANOVA and for effects of mycorrhiza and species identity

using a one-way ANOVA, respectively. Similarly, two-way ANOVA was performed on MGD (factors: species identity and competition), root/shoot ratio (factors: fertilization level and species identity) and RNE (factors: fertilization level and mycorrhiza) data. Effects of species identity, competition and fertilization level on the degree of mycorrhizal colonization were assessed by three-way ANOVA on all AM plants. When significant differences were found for main effects, Fisher's LSD post-hoc pair wise comparison was applied to determine individual differences between means.

2.4 Results

2.4.1 Species-specific traits (single plants)

Mycorrhizal colonization of the NM plants was zero in all cases, whereas AM plants of both species revealed high levels of colonization (ranging from 84 to 94 %; Fig. 2.1). Colonization values of *H. pilosella* single plants were marginally higher than those of the corresponding *P. lanceolata* plants at the fertilization levels 0.25 and 0.5. At the highest nutrient level

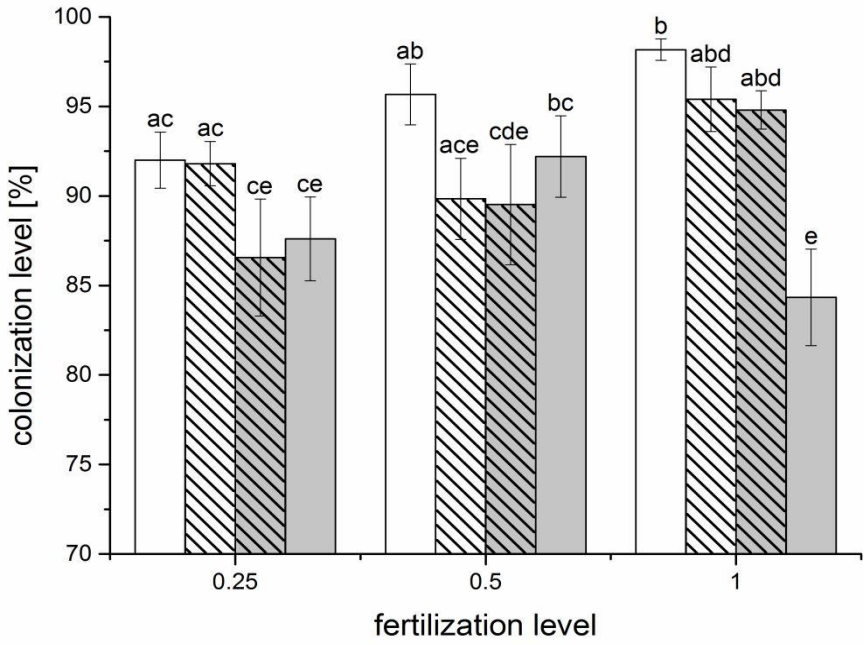

Fig. 2.1 Colonization levels of AM plants of *H. pilosella* and *P. lanceolata*. Values are shown for *H. pilosella* single plants (white bars), *H. pilosella* competition plants (white, hatched bars), *P. lanceolata* single plants (grey bars) and *P. lanceolata* competition plants (grey, hatched bars). Different letters indicate significant differences at p = 0.05 (ANOVA). Means ± SE, n = 6.

H. pilosella single plants were almost completely colonized (98%) while colonization of *P. lanceolata* was significantly ($p < 0.001$) lower (84%).

Mycorrhization led to a significant ($p < 0.001$) biomass increase in the single plants of both species (Fig. 2.2). In all fertilizer treatments and independent of mycorrhization biomass production of *P. lanceolata* single plants was significantly ($p < 0.01$) higher than in *H. pilosella* (Fig. 2.2). NM single plants of *P. lanceolata* produced between five (fertilization

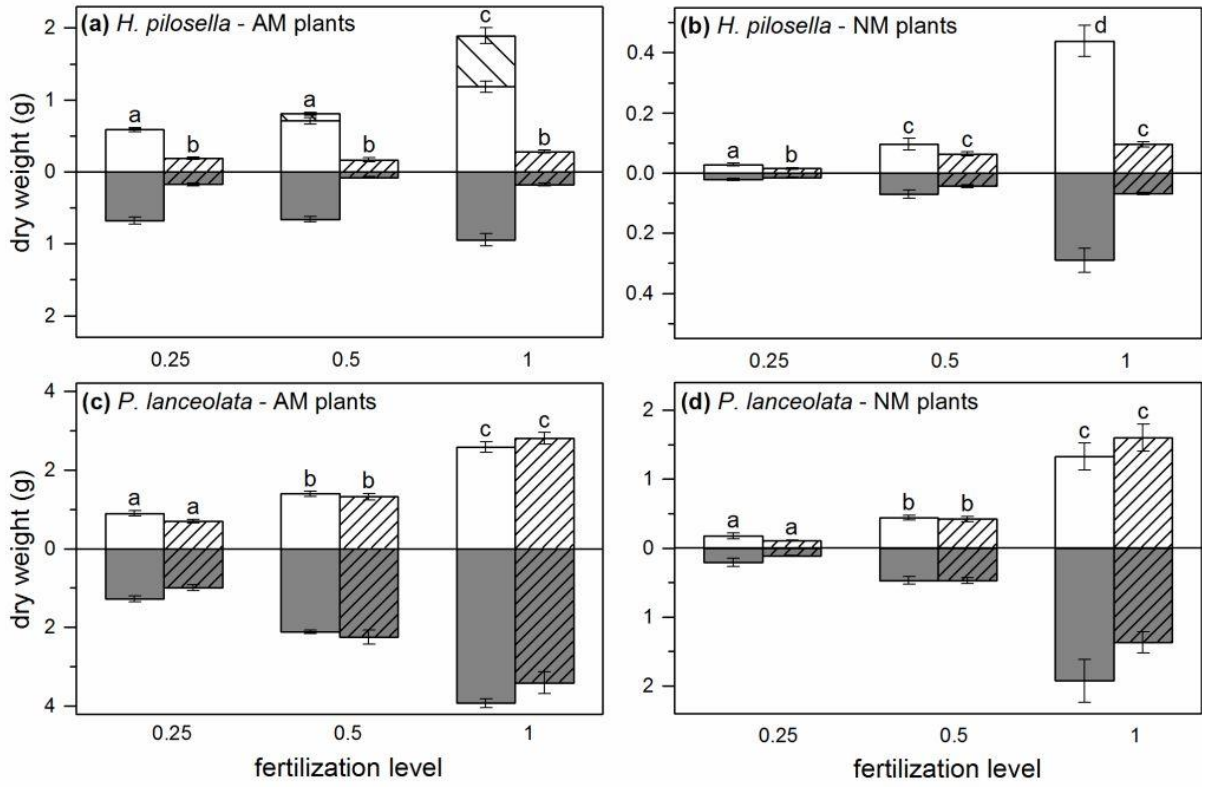

Fig. 2.2 Dry weight of roots (lower bars) and shoots (upper bars) for single plants (open bars) and competition plants (hatched bars). (a) AM *H. pilosella* (stolon dry weight is shown as coarse hatched bars on top of the upper bars), (b) NM *H. pilosella*, (c) AM *P. lanceolata*, (d) NM *P. lanceolata*. Note different scaling! Different letters indicate significant differences between total dry weights at $p = 0.05$ (ANOVA). Means ± SE, $n = 6$.

levels 0.5 and 1) and eight (fertilization level 0.25) times more biomass than NM single plants of *H. pilosella* (Fig. 2.2b, d). Mycorrhization lowered these interspecific biomass differences (Fig. 2.2a, c), with *P. lanceolata* single plants only reaching approx. 2.5 times the biomass of *H. pilosella* single plants at the fertilization levels 0.5 and 1. Interestingly, the smallest difference in total dry weight between the two species was observed at the lowest fertilization level, where *P. lanceolata* exhibited only 1.7 times the biomass of *H. pilosella* (Fig. 2.2a, c).

Reducing the fertilization from level 1 to 0.5 led to significant differences ($p < 0.001$) in total dry weight in all single plants and particularly in the NM plants with reductions of

77% in *H. pilosella* and 72% in *P. lanceolata* (Fig. 2.2b, d). The corresponding AM plants exhibited a lower decrease of 48% and 46%, respectively. Lowering fertilization levels from 0.5 to 0.25 led to further biomass reductions, with NM plants showing a stronger decrease (*H. pilosella*: -70%; *P. lanceolata*: -58%) than AM plants, where *P. lanceolata* showed a 38% reduction while, most interestingly, *H. pilosella* exhibited no response ($p = 0.235$).

At all fertilization levels *H. pilosella* single plants showed a significantly ($p < 0.001$ at fertilization level 0.25 and 1; $p < 0.05$ at fertilization level 0.5) higher MGD than *P. lanceolata*

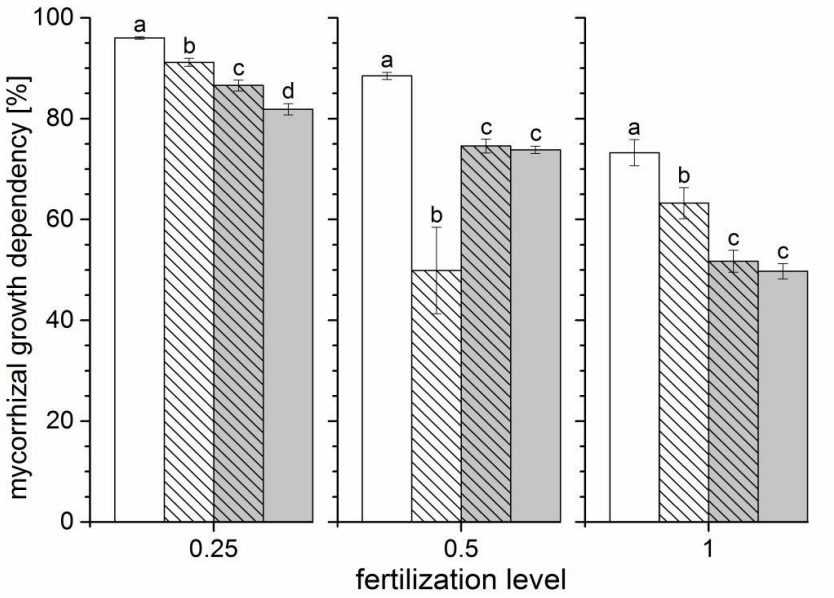

Fig. 2.3 Mycorrhizal growth dependency (MGD) for *H. pilosella* single plants (white bars), *H. pilosella* competition plants (white, hatched bars), *P. lanceolata* single plants (grey bars) and *P. lanceolata* competition plants (grey, hatched bars). Different letters indicate significant differences within each fertilization level at $p = 0.05$ (ANOVA). Means ± SE, $n = 6$.

(Fig. 2.3). MGD of both species was highest at fertilization level 0.25, with *H. pilosella* and *P. lanceolata* exhibiting values of 96% and 82%, respectively. While *H. pilosella* showed a MGD of 88% and 73% in fertilization levels 0.5 and 1, the respective values of *P. lanceolata* were only 74% and 50%. The MGD values of both species decreased unproportionally with increasing fertilization, which led to the highest interspecific MGD difference in fertilization level 1.

P. lanceolata single plants had significantly higher ($p < 0.01$) r/s-ratios than the corresponding *H. pilosella* plants, with interspecific differences markedly increasing with nutrient availability (Fig. 2.4). In the AM treatment, however, *P. lanceolata* did not change its high r/s-ratio with increasing nutrient availability (Fig. 2.2c, 2.4a), whereas *H. pilosella* significantly ($p < 0.01$) lowered its r/s-ratio with increasing fertilization level. As shown in

Fig. 2.2 this was caused by a proportional increase of root and shoot biomass in *P. lanceolata*, whereas *H. pilosella* only increased shoot biomass. In contrast to the AM treatment, NM *H. pilosella* showed no r/s-ratio decrease with increasing fertilization level, whereas the corresponding NM *P. lanceolata* increased root biomass stronger than shoot biomass (Fig. 2.2d, 2.4b). Biomass allocation to reproductive organs was only observed in *H. pilosella* AM plants, where stolons accounted for 7 and 25% of total dry weight at fertilization levels 0.5 and 1, respectively (Fig. 2.2a).

2.4.2 Competitive interactions between both species

At fertilization levels 0.25 and 0.5, colonization levels of both plant species were not significantly affected by competition (Fig. 2.1). At fertilization level 1, however, colonization of *P. lanceolata* was significantly increased from 84 to 95% ($p < 0.01$), when *H. pilosella* was present, while *H. pilosella* remained unaffected.

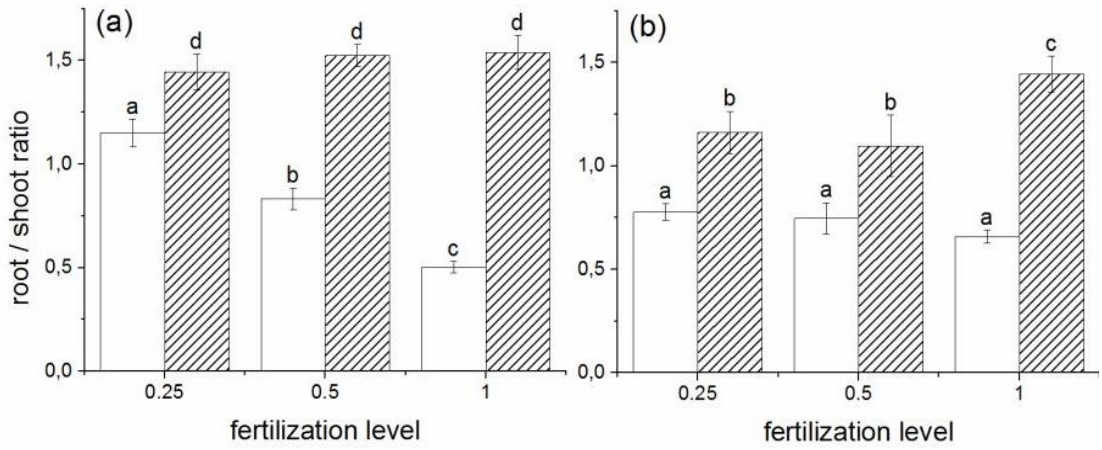

Fig. 2.4 Root/shoot ratios of AM plants (a) and NM plants (b) for *H. pilosella* (open bars) and *P. lanceolata* (hatched bars), respectively. Different letters indicate significant differences at $p = 0.05$ (ANOVA). Means ± SE, $n = 6$.

Competition between the two species was detected in AM as well as NM treatments at all fertilization levels with *P. lanceolata* clearly being the stronger competitor as indicated by the significant growth reduction of *H. pilosella* when competing with *P. lanceolata* (Fig. 2.2a, b). In contrast, *H. pilosella* did not significantly affect the growth of *P. lanceolata* at fertilization levels 0.5 and 1. However, at fertilization level 0.25 *H. pilosella* induced a slight, although non-significant, growth reduction in *P. lanceolata* (Fig. 2.2c, d). A comparison of RNE-values between AM and NM treatments revealed that *P. lanceolata*

exerted a significant competitive pressure on *H. pilosella*, both in presence and absence of AMF (Fig. 2.5). However, mycorrhization induced a significant amplification of the competitive imbalance in fertilization levels 0.25 and 0.5, as indicated by increased RNE values for *H. pilosella* (Fig. 2.5a) and decreased values for *P. lanceolata* (Fig. 2.5b). The

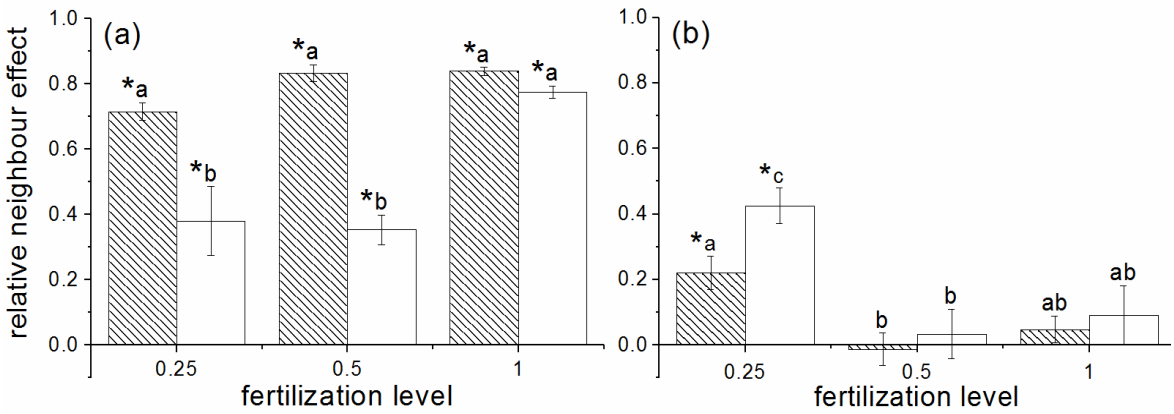

Fig. 2.5 Relative neighbor effect on *H. pilosella* (a) and *P. lanceolata* (b) for AM plants (hatched bars) and NM plants (open bars), respectively. Different letters indicate significant differences between RNE values at p = 0.05 (ANOVA). Values with asterisks are significantly different from zero at p = 0.05 (t-test against zero). Means ± SE, n = 6.

strongest competitive pressure on *H. pilosella* NM plants was detected at fertilization level 1. However, the amplification of this effect due to the presence of AMF, occurring at the lower fertilization levels, was not observed here (Fig. 2.5a).

MGD of *H. pilosella* was significantly reduced by competition at all fertilization levels ($p < 0.001$ at fertilization levels 0.25 and 0.5; $p < 0.01$ at fertilization level 1; Fig. 2.3). In contrast, MGD of *P. lanceolata* was not reduced by competition. However, most interestingly, at fertilization level 0.25 the beneficial mycorrhizal effects on *P. lanceolata* were significantly amplified by the presence of *H. pilosella* ($p < 0.01$; Fig. 2.3).

2.5 Discussion

2.5.1 Species-specific traits and foraging strategies

The results from the single plants confirm our assumption that *H. pilosella* and *P. lanceolata* differ in their foraging strategies regarding AMF-mediated vs. root-mediated nutrient acquisition. While *P. lanceolata* showed a considerably high biomass allocation to its root system (see also Kutschera & Lichtenegger 1992), *H. pilosella* allocated only a small proportion of biomass belowground (Bishop & Davy 1994). Further, we found clear species-specific

differences in mycorrhizal responsiveness: *H. pilosella* revealed to be highly mycotrophic, as previously reported by Grime *et al.* (1987), exhibiting extraordinarily large mycorrhizal benefits (MGD close to 100%) under low nutrient availability, which is in line with the findings of van der Heijden *et al.* (1998) suggesting *H. pilosella* to be obligatory mycotrophic. As obligatory mycotrophic plants are completely dependent on mycorrhiza over the range of soil fertility they naturally encounter (Janos 1980), *H. pilosella* is expected to acquire nutrients mainly via AMF. This is supported by the fact that AM *H. pilosella* showed no increase in root biomass with increasing nutrient availability (Fig. 2.2a). Obviously, the majority of nutrient depletion can in this case be attributed to the AMF and additional root growth might have been waste of resources (Schweiger *et al.* 1995). Possibly, enlarging the root system with rising nutrient availability was not necessary because root density was already optimized for a minimal overlap of root and hyphal nutrient depletion zones (Vance *et al.* 2003; Jakobsen *et al.* 2005) and for creating an adequate interface between plant and fungus, thereby ensuring a maximum rate of nutrient exchange. Interestingly, AM *H. pilosella* single plants growing under increased nutrient availability allocated a considerable proportion of biomass to clonal growth (stolons) instead of the root system. This finding is consistent with the formation of aggregations by clonal growth, often observed for *H. pilosella* in its natural habitats (Bishop & Davy 1994).

As would be expected from its more root-mediated foraging strategy *P. lanceolata* was less responsive to mycorrhization than *H. pilosella*. However, this comparatively smaller growth response of *P. lanceolata* to mycorrhiza is still relatively high as compared to other studies, where negative, neutral or only slightly positive responsiveness was detected (Gange & West 1994; Parádi *et al.* 2003; Ayres *et al.* 2006; Heinemeyer *et al.* 2006; Scheublin *et al.* 2007). The clearly pronounced positive responsiveness of both species in our experiment was probably promoted by low nutrient availability and the relatively large pot volume per plant (Janos 2007; Johnson 2010). Furthermore, responsiveness to mycorrhization has been shown to be inversely correlated with plant density (Hartnett *et al.* 1993; Facelli *et al.* 1999; Schroeder & Janos 2004; Janos 2007; Schroeder-Moreno & Janos 2008). In a high density setup with *P. lanceolata,* Scheublin *et al.* (2007) even found a null-responsiveness to mycorrhization. Thus, the low density of one or two plants per pot in the present experiment might be a likely explanation for the comparatively high responsiveness observed for *P. lanceolata*.

2.5.2 Relevance of the different foraging strategies in competitive interactions

There is a number of potential advantages of AMF-mediated foraging over root-mediated foraging, like expansion of the depletion zone by mycorrhizal hyphae (Jakobsen *et al.* 2005; Smith & Read 2008), lower C-costs for production of hyphal absorption surface area compared to root surface area (Fitter 1991; Jakobsen *et al.* 1992; Schweiger *et al.* 1995) and a higher phosphorus use efficiency (i.e. the efficiency, by which acquired phosphorus is reinvested to acquire more phosphorus; Koide *et al.* 2000). Nevertheless, the question to what extent AMF-mediated foraging can serve as an effective competition mechanism as compared to root-mediated foraging is still controversial (e.g., Allen & Allen 1984; Hartnett *et al.* 1993; Smith *et al.* 1999; Scheublin *et al.* 2007; Daisog *et al.* 2012).

In absence of AMF *P. lanceolata* was the dominant competitor, while growth of *H. pilosella* was markedly suppressed, which is in accordance with our first hypothesis. With any mycorrhizal effects on competition ruled out in the NM plants, this competitive imbalance is explained by the relatively large root system of *P. lanceolata*, as size and architecture of the root system have been shown to be one of the most important factors determining belowground competitive strength (Casper & Jackson 1997; Weiner *et al.* 1997; Hodge *et al.* 1999; Cahill & Casper 2000; Fitter *et al.* 2002; Weigelt *et al.* 2007). Interestingly, the competition-related growth suppression of NM *H. pilosella* decreased with decreasing nutrient availability with both species exerting an equal competitive pressure on each other at the lowest fertilization level. This was unexpected because the relative difference in belowground biomass between single plants of *P. lanceolata* and *H. pilosella* was highest under these conditions (Fig 2.2b, d). Root competition has been shown to be size symmetric (Weiner 1986; Weiner *et al.* 1997; Cahill & Casper 2000; Bartelheimer *et al.* 2008). Therefore, we expected a larger competitive imbalance in favor of *P. lanceolata* in the low fertilization level. The relatively high competitive ability of *H. pilosella* under low nutrient availability, independent of AM, was possibly caused by a generally better adaptation to nutrient deficient habitats (e.g., by higher nutrient uptake efficiency of the roots) as compared to *P. lanceolata* (Ellenberg 1974; Kutschera & Lichtenegger 1992; Bishop & Davy 1994).

Since the highly mycotrophic *H. pilosella* was found to be more responsive to mycorrhizal infection and gained considerably higher benefits from the association with the fungus than *P. lanceolata*, we expected the competitive relationship to be altered in favor of *H. pilosella* in the presence of AMF. In contrast to this hypothesis, presence of AMF significantly amplified the competitive imbalance in favor of *P. lanceolata*, under low and intermediate

nutrient availability, as compared to the NM treatment. Under high nutrient availability, however, we observed only a marginal mycorrhiza-mediated amplification of the competitive pressure by *P. lanceolata* (Fig. 2.5). These results indicate that the AMF-mediated foraging strategy of *H. pilosella* is obviously not a suitable competition mechanism in presence of *P. lanceolata.* This is in contrast to some other studies, where presence of mycorrhiza altered competition in favor of the most mycotrophic species (e.g., Allen & Allen 1984; Hartnett *et al.* 1993; Smith *et al.* 1999; Scheublin *et al.* 2007). One reason might be that in those studies highly mycotrophic species were compared with weakly or null-responsive plants, whereas in our experiment the putatively low responsive *P. lanceolata* received considerable mycorrhizal benefits, thus diminishing the AMF-mediated advantage of *H. pilosella*. Another, perhaps more important, reason for the poor competitive performance of *H. pilosella* might be the much bigger root system of *P. lanceolata*. Although, as mentioned before, there are several advantages of AMF-mediated foraging, there is one important advantage of root-mediated foraging: The root system of a plant is an exclusive nutrient acquisition organ, as nutrients, once acquired from the soil, cannot be taken up by neighboring plants (de Kroon *et al.* 2003; Lynch 2005). In contrast, the extraradical AMF mycelium is an acquisition organ, which is often not exclusive due to the development of CMNs (Leake *et al.* 2004; van der Heijden & Horton 2009), interlinking several host plants. While in a monoxenic system the major part of nutrients, acquired by AMF, can be used by one single host plant, mycotrophic plants in a multixenic system are forced to compete for the nutrients acquired by the CMN (Newman *et al.* 1992). Hence, the suitability of AMF-mediated foraging for exerting competitive pressure might depend on the degree of mycotrophy of the competitor. In other words, AMF-mediated foraging should function most efficiently in an interaction with a non-mycotrophic competitor (Allen & Allen 1984) because in that case nutrient acquisition via AMF is exclusive, and AMF-mediated competitive strength should decrease with the degree of mycotrophy of the competitor. Thus, in our experiment the potential AMF-mediated competitive strength of *H. pilosella* was diminished by the relatively high degree of mycotrophy of *P. lanceolata*.

Furthermore, facilitation of *P. lanceolata* by *H. pilosella* via CMN cannot be ruled out. Some studies showed that the symbiosis between AMF and host plant is stabilized by physiological mechanisms, bidirectionally controlling the reciprocal exchange of nutrients (e.g., Kiers *et al.* 2011), thus making ‘cheating’ between both partners unlikely. However, it is yet unknown if cost/benefit ratios of plants interconnected by a CMN, are always proportional (van der Heijden & Horton 2009). As the highly mycotrophic *H. pilosella* invests high amounts of carbon into the AMF, *P. lanceolata* being connected via CMN, could possibly

invest less carbon into the fungal symbiosis and, thus, benefit from the presence of *H. pilosella*. This is supported by the fact that at the highest fertilization level mycorrhizal colonization of *P. lanceolata* was significantly increased in the presence of *H. pilosella* (Fig. 2.1). Furthermore, we observed a significantly increased MGD in *P. lanceolata* due to presence of *H. pilosella* at the 0.25 fertilization level (Fig. 2.3). Moreover, at the higher fertilization levels, *P. lanceolata* did not show any growth suppression in presence of *H. pilosella* although this should have been expected in view of the relatively high biomass of the corresponding *H. pilosella* single plants (Fig. 2.2a, c). The lack of growth suppression in *P. lanceolata* might indicate that competitive effects of *H. pilosella* were neutralized by facilitative effects via CMN.

In marked contrast to *P. lanceolata*, *H. pilosella* produced a relatively low root biomass, which did not increase with increasing nutrient availability, resulting in low root-mediated competitive strength. On the other hand, we found a clear allocation trade-off in

Fig. 2.6 Clonal dominance stand of *Hieracium pilosella*. Photograph taken at the Hainberg Reserve near Nürnberg, Germany.

favor of clonal reproductive biomass at the expense of root growth. We suggest that in terms of carbon investment the AMF-mediated foraging strategy is cheaper than root growth (Fitter 1991; Jakobsen *et al.* 1992; Schweiger *et al.* 1995) and therefore allows for enhanced C allocation into vegetative reproduction in order to form the well known closed clonal aggregations of *H. pilosella* at the community scale in the field (Bishop *et al.* 1978; Widera 1978; Bishop & Davy 1994, also see Fig. 2.6), which minimize interspecific competition (Tilman 1988). However, although this allocation pattern might provide a competitive advantage on the community scale, the AMF-mediated foraging strategy appeared unsuitable to act as an effective direct belowground competition mechanism.

Although *P. lanceolata* dominated the competitive relationship in all cases (except fertilization level 0.25 in the NM treatment, where competition was balanced), the competitive interactions were markedly influenced by nutrient availability. While at intermediate and high fertilization levels AM *H. pilosella* experienced high competitive pressure and was not able to affect growth of *P. lanceolata*, this competitive imbalance was mitigated in favor of *H. pilosella* in the low fertilization treatment (Fig. 2.5). Nevertheless, our third hypothesis has to be rejected, as not only AM *H. pilosella*, but also the corresponding NM plants showed the best competitive performance under low nutrient availability. Thus, it is not clear to what extent the decrease of the competitive imbalance under nutrient deficiency was mediated by AMF or whether it was merely a result of a generally worse adaption of *P. lanceolata* to nutrient deficiency as compared to *H. pilosella*. Moreover, AM *H. pilosella* exhibited its highest root/shoot-ratio at the lowest fertilization level, indicating an increased root-mediated competitive strength. However, since the NM single plants of both species as well as the AM single plants of *P. lanceolata* reduced their biomass significantly between the fertilization levels 0.5 and 0.25, while interestingly, only the corresponding AM *H. pilosella* showed no growth reduction (Fig. 2.2), a mycorrhiza-mediated positive influence on the competitive performance of *H. pilosella* under nutrient deficiency can not be excluded. However, our results emphasize the importance of taking nutrient availability into account when trying to disentangle the role of mycotrophy in competitive interactions.

As previously mentioned, studies that found shifting competitive interactions in favor of highly mycotrophic species as a consequence of mycorrhization, often used experimental setups with high plant densities (e.g., Grime *et al.* 1987; Hetrick *et al.* 1994; Scheublin *et al.* 2007). This might, however, cause a nutrient limitation sufficient to induce not only a relative (as observed in our experiment at the lowest fertilization level) but also an absolute shift of competitive interactions in favor of the most mycotrophic species. Therefore it is important to rule out density related competition effects to further analyze the role of mycorrhizae in competitive relationships.

2.6 Conclusions

Our study showed that AMF-mediated foraging can be a less effective competition mechanism than root-mediated foraging. Nevertheless, the high mycorrhizal benefits gained by the obligatory mycotrophic plant *H. pilosella* suggest that this strategy enables a rapid establishment of closed aggregations by clonal growth at the community level to reduce interspecific competition in its natural habitat. However, for direct belowground competition

between two mycotrophic plants, root-mediated foraging seems to be a more effective competition mechanism than AMF-mediated foraging, as nutrient acquisition via roots is always exclusive while CMN-acquired nutrients have to be shared between the competitors. Moreover, our results emphasize the high importance of root parameters for the interpretation of competitive interactions and their relation to foraging strategies. Similarly, plant density and nutrient availability have to be taken into account to assess the outcome of interspecific competition as affected by different degrees of mycotrophy. Understanding the relative importance of AMF- vs. root-mediated foraging strategies is crucial for disentangling the effects of arbuscular mycorrhiza on plant-plant interactions and the related successional processes.

2.7 Authors contributions

I designed the experiment and accomplished the entire experimental work, data analysis, and writing of this chapter. Further contributions were given by S. Unger, C. Werner and W. Beyschlag, who assisted with data interpretation and writing. Further, I acknowledge D. Behringer, E. Furlkröger, S. Kindermann, H. Landskron and B. Teichner for support with plant cultivation and laboratory work and the workgroup of Prof. M. Rillig for valuable methodological input.

CHAPTER 3

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Experiment 2

Potential advantages of highly mycotrophic foraging for the establishment of early successional pioneer plants on sand

3.1 Abstract

Adaptive traits ensuring efficient nutrient acquisition, such as extensive fine root systems, are crucial for establishment of pioneer plants on bare sand. Some successful pioneer species of temperate, European sand ecosystems are characterized as obligate mycorrhizals, thus likely substituting fine roots by AMF. However, it is not clear, if AMF-mediated acquisition of scarce and immobile nutrients such as P is an advantageous strategy on bare sand, compared to foraging via roots. We compared the foraging performance of three obligately mycorrhizal forbs and two facultatively mycorrhizal grasses, regarding the influence of AMF on their capacity to acquire P from bare sand. Comparison of mycorrhizal and non-mycorrhizal individuals revealed a markedly higher AMF-dependency for P acquisition and growth in the forbs than in the grasses. Periodical soil core sampling, allowing for assessment of root and hyphal growth rates, revealed hyphal growth to markedly enlarge the total absorptive surface area (SA) in the forbs, but not in the grasses. Correlations between SA growth and P depletion suggest an AMF-induced enhanced capacity for rapid soil P exploitation in the forbs. Our study showed that AMF-mediated foraging may be an advantageous strategy over root-mediated foraging in sand pioneer plants.

3.2 Introduction

Mechanisms and traits for efficient exploitation of limiting resources are of major importance for the competitive success of plants and thus for vegetation dynamics and successional progress (Tilman 1985). In the early successional stages of temperate, open sand ecosystems, where plant density is low (i.e. minor limitations due to shading) and soils are not yet or only poorly developed, belowground resources such as water and nutrients are typically the main limiting factors for plant growth (Boorman 1982; Weigelt *et al.* 2005). Pioneer plant species, which are able to successfully establish on bare sand, are of particular interest when studying adaptations to nutrient deficiency (Olff *et al.* 1993; Bartelheimer *et al.* 2006; Weigelt *et al.*

2007; Le Bagousse-Pinguet *et al.* 2013). It is well known, that high fine root proportions are an appropriate trait for the acquisition of scarce and immobile soil nutrients, such as P (Ryser & Lambers 1995; Lambers *et al.* 2008; Richardson *et al.* 2009). Thus, perennial grasses with pronounced extensive fine root systems, such as *Corynephorus canescens* (Kutschera & Lichtenegger 1982; Bartelheimer *et al.* 2006), are predisposed to colonize bare sand and often dominate the early successional stages of temperate, open sand ecosystems (Ellenberg 1996; Jentsch & Beyschlag 2003). While most studies on the foraging performance of sand pioneer plants focus on root morphological traits (e.g., Weigelt *et al.* 2007; Bartelheimer *et al.* 2008), less attention has been given to alternative ways of nutrient acquisition. In this regard, the association with AMF, which may be employed by 80% of all terrestrial plants including 74% of all Angiosperms (e.g., Brundrett 2009), has been revealed as a quite successful substitute for extensive fine root systems (Baylis 1975; Brundrett 2002). Indeed, several successful early colonizers in open sand ecosystems are characterized as obligately mycorrhizal (Wang & Qiu 2006), but it is not clear to which extent their success can be related to their highly mycotrophic lifestyle. However, it has been suggested that the production of belowground absorptive SA by mycorrhizal hyphae may be much 'cheaper' in terms of C allocation than the production of an equal root SA (Harley 1989; Fitter 1991), which might enable faster SA growth in plants with a more AMF-mediated foraging strategy. Hence, foraging via mycorrhizal hyphae might be advantageous as compared to foraging via roots in nutrient-poor open sand ecosystems, where rapid exploitation of bare sand patches, which are frequently evolving due to erosion and disturbance (Jentsch & Beyschlag 2003), might be a key trait for species success (Casper & Jackson 1997; Grime 2001).

In order to clarify this assumption, we compared the belowground invasion of bare sand by root and hyphal SA production and the related P exploitation capacity of three obligately mycorrhizal forbs (*Hieracium pilosella*, *Hypochaeris radicata* (Asteraceae) and *Plantago lanceolata* (Plantaginaceae)) and two facultatively mycorrhizal grasses (*Corynephorus canescens* and *Festuca psammophila* (Poaceae)) (Wang & Qiu 2006). All five species frequently co-exist in the early successional stages of European temperate, open sand ecosystems and are often found to colonize bare sand (Ellenberg 1996; Jentsch & Beyschlag 2003). Besides estimations on the species-specific AMF-dependency, we tried to disentangle potential advantages of AMF-mediated over root-mediated foraging in uncolonized sand patches.

We hypothesized that (1) in contrast to the facultatively mycorrhizal grasses, P uptake and growth of the obligately mycorrhizal forbs are strongly AMF-dependent and that (2) forbs

and grasses differ in their specific C allocation trade-off between the production of root and hyphal SA, with a predominantly root-directed allocation in the grasses and a predominantly AMF-directed allocation in the forbs. Finally, we tested the hypothesis, that (3) a predominantly AMF-directed C allocation leads to higher total SA growth rates, thus providing an advantage in terms of P depletion from bare sand patches to the forbs.

3.3 Materials and methods

3.3.1 Plant cultivation

We performed a controlled pot experiment using AM and NM individuals of *Hieracium pilosella* L., *Plantago lanceolata* L., *Hypochaeris radicata* L., *Corynephorus canescens* (L.) P. BEAUV. and *Festuca psammophila* (HACK. EX ČELAK.) FRITSCH in a growth chamber at a light (photosynthetic photon flux density of approx. 320 µmol m^{-2} s^{-1}) / dark period of 14 h / 10 h, a temperature of 22°C / 15°C and a relative air humidity of 65%. Seeds (Blauetikett-Bornträger GmbH, Offstein, Germany; *Botanical Garden* of the Westfälische Wilhelms-Universität *Münster*, Germany) were sown and started in boxes with sand ('Wesersand', grain size 0.063 - 2 mm, pH = 7.3; plant-available phosphate-P = 0.49 mg kg^{-1}; plant-available nitrate-N = 1.74 mg kg^{-1}) which was previously sterilized in an autoclave (FVA/A1, Fedegari, Switzerland) for 1.5 h at 120°C. Two weeks after germination, the seedlings were transplanted into small pots (100 cm^3). Eight plants of each species (AM treatment) were inoculated using an inoculum-sand-mixture (*Rhizophagus intraradices*, (N.C. SCHENCK & G.S. SMITH) C. WALKER & A. SCHÜßLER, INOQ GmbH, Schnega, Germany), while six plants of each species (NM treatment) received sterilized sand. In order to create a comparable soil microbial community, NM plants received 5 ml of a microbial wash, which was extracted from the inoculum by sieving the supernatant of a water-inoculum-mixture through a 20 µm sieve (Koide & Li 1989). AM plants received 5 ml of deionized water instead. After eight weeks of growth, plants were transferred to larger pots (3,000 cm^3) for another 12 weeks of growth until final harvest.

Plants were supplied with deionized water according to demand to keep soil moisture at approx. 6%. Twice a week a modified Hoagland fertilizer solution (Hoagland & Arnon 1950) was applied. During the experiment, fertilizer volume and concentration were increased from 3 to 5 ml and from a dilution of 1:4 over 1:2 to full concentration (3 mmol KNO_3, 1 mmol $Ca(NO_3)_2$, 0.5 mmol $(NH_4)_2SO_4$, 0.5 mmol $(NH_4)_2HPO_4$, 1 mmol $MgSO_4$, 0.5 mmol KCl, 0.5 mmol $FeC_6H_5O_7$, 0.0125 µmol H_3BO_3, 0.001 µmol $MnSO_4$, 0.001 µmol $ZnSO_4$,

0.00025 µmol $CuSO_4$, 0.00025 µmol MoO_3; per liter), to adjust the application to the increasing nutrient demand of the plants. Once a week, the pot positions were randomized to rule out edge and location effects. None of the plants reached the reproductive state and all appeared healthy throughout the experiment.

3.3.2 Determination of root and hyphal surface area growth rates

At plant ages of 12, 14, 16 and 18 weeks, soil cores were extracted at four symmetrically distributed spots from each pot using a cylindrical steel borer with a diameter of 2.7 cm, reaching from the top of the substrate to the bottom of the pot, resulting in a total extraction volume of 485 cm^3 per pot. The four samples of each pot were then united, thoroughly mixed and root-free subsamples were separated and dried at 40°C for analyses of hyphal density (AM treatment only) and soil P concentration (see section 3.3.3). All roots were collected from each sample by sieving and washing, scanned at 600 dpi and analyzed using WinRhizo Pro (Version 2003 b, Regent Instruments Inc., Quebec, Canada) in order to determine the recently produced root SA per soil dry weight and the species-specific distribution of root size classes, with particular focus on the proportion of fine roots (diameter < 0.2 mm). Subsequent to each soil core extraction, the holes were refilled with sterilized sand. Soil core samplings were taken using a template that was aligned to marks on the pot rim, thus ensuring insertion of the steel borer at exactly the same spots at each soil core extraction. A vertical passage in the template matching the diameter of the steel borer enabled insertion in a constant angle of 90 ° (Fig. 3.1).

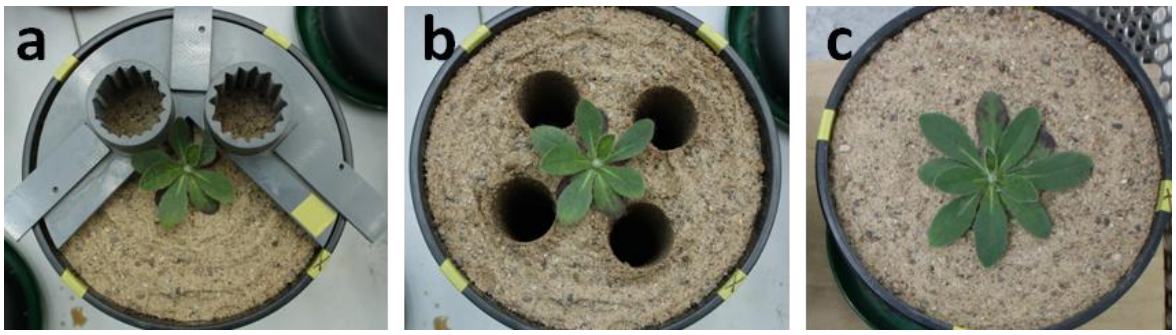

Fig. 3.1 Soil core sampling procedure: (a) Aligning a template to marks on the pot rim, (b) extracting four soil cores using a steel borer, and (c) refilling holes with fresh substrate.

Extraradical hyphae were quantified in all AM plants, utilizing an aqueous extraction and membrane filter technique adapted from Jakobsen *et al.* (1992). Twenty g of dried substrate were suspended in a solution of 100 ml deionized water and 12 ml sodium

hexametaphosphate solution (35 g l^{-1}), vigorously shaken for 30 s. After 1 h, the suspension was transferred to a 40 µm sieve. The material on the sieve was rinsed gently with deionized water to remove clay particles and transferred to a 250 ml Erlenmeyer flask which was subsequently filled with 200 ml deionized water. The flask was shaken thoroughly for 5 s to flotate the hyphae. After 60 s, an aliquot of 10 ml was taken from a defined height of the supernatant and drawn through a 25 mm membrane filter (0.45 µm pore size). Fungal material on the filter was specifically stained with a Trypan Blue solution (5:1 = (2:1:2 = lactic acid : glycerin: H_2O) : Trypan Blue (0.4%, Sigma-Aldrich Chemie GmbH, Germany)) for 5 min. After rinsing with deionized water, the filter was transferred to a microscope slide and hyphal density expressed as length per soil dry weight was determined according to Miller *et al.* (1995) at x 250 magnification. Additionally, the average hyphal diameter was measured at x 400 magnification for one representative sample of each species and then used for calculation of the recently produced hyphal SA per soil dry weight (*A*, Eq. 3.1).

$$A = R \cdot 2\pi r \qquad \text{Eq. 3.1}$$

, where *R* is the hyphal length per soil dry weight and *r* is the radius of hyphae. As the hyphal diameter did not differ significantly between the five plant species (data not shown), the average diameter (4.7 µm; *r* = 2.35 µm) was induced as a constant in all calculations.

3.3.3 Determination of soil P depletion rates

Plant available phosphate-P in the soil core samples was extracted using a modified calcium-acetate-lactate (CAL) extraction method according to Schüller (1969). A suspension of 5 g of dried substrate and 50 ml CAL solution (77 g calcium lactate, 39.5 g calcium acetate, 89.5 ml 100% acetic acid l^{-1}) was shaken for 90 min, then centrifuged at 3000 rpm for 3 min and the supernatant was passed through a glass fiber filter (1 µm pore size) using a Luer syringe. Orthophosphate concentration in the extract was measured colorimetrically at 880 nm using flow injection analysis (FIA-Lab II, MLE GmbH, Dresden, Germany). The sand used for refilling the holes contained plant available phosphate-P in a concentration of 1.9 mg kg^{-1}. This value was set as initial concentration for the calculation of 14-day P depletion rates. The added fertilizer solution (see section 3.3.1) was considered in these calculations.

3.3.4 Quantification of mycorrhizal root colonization

During the fourth soil core sampling, representative subsamples of the extracted roots of both, AM and NM plants were analyzed for mycorrhizal colonization. The roots were bleached in 10 % KOH at 90°C for 10 min, rinsed with deionized water and stained with an ink-acetic-acid solution (1:1:8 = ink : 10% acetic-acid : H_2O) at 90°C for 15 min, followed by a final, intense rinsing with deionized water (Phillips & Hayman 1970). The root fragments were then transferred to microscope slides and the percentage of root length colonized by AMF was estimated at x 250 magnification using a modified intersect method (McGonigle *et al.* 1990), scoring a minimum of 100 intersections per sample for the presence of hyphae, vesicles and arbuscules.

3.3.5 Assessment of mycorrhizal growth dependency and plant tissue P concentration

After 20 weeks of growth, all plants were harvested and separated into above- and belowground material. After cleaning roots from substrate, both root and shoot material was dried at 60°C and weighed. Total dry weight of AM and NM plants was used for calculation of the species-specific MGD (Eq. 3.2), according to Smith *et al.* (2003):

$$MGD = 100\frac{AM - \overline{NM}}{AM} \qquad \text{Eq. 3.2}$$

, where *AM* is the dry weight of an individual AM plant and $\overline{NM}$ is the mean dry weight of the corresponding NM plants. This index is based on the equation of Plenchette *et al.* (1983), resulting in values ranging from -∞ to +100%, but was further adapted according to Gange and Ayres (1999), allowing for calculation of variance as MGD values can be quantified for individuals.

Plant P content was measured for root and shoot tissues using high-temperature oxidation and colorimetrical quantification according to Watanabe & Olsen (1965). Dried plant material was ashed at 500°C for 4 h in a muffle furnace and, after cooling, 7 mg of ash was digested in 10% nitric acid. The extracts were diluted with bidestilled water and analyzed for orthophosphate concentration using FIA, as described above. Tissue P concentration was calculated by relating the results to plant dry weight. Plant material of NM *H. pilosella* individuals had to be pooled in order to reach a sufficient quantity of ash for analysis.

3.3.6 Statistical analyses

Statistical analyses were performed using SigmaPlot 11.0 (2008, SYSTAT SOFTWARE, INC., Chicago, USA). Data were tested for normal distribution (Shapiro-Wilk test) and homogeneity of variances (Brown-Forsythe test). Data that did not satisfy the assumptions of normal distribution were square root or log transformed prior to analysis. One-way ANOVA was performed on data of MGD, colonization level, abundance of arbuscules and vesicles as well as on root and hyphal absorptive SA growth rates (factor: species). Two-way ANOVA was performed on fine root proportions, total biomass, total absorptive SA growth rates, P depletion rates within each ingrowth period and tissue P concentration (factors: species and mycorrhization). When significant differences were found for main effects, Fisher's LSD post-hoc pair wise comparison was applied to determine individual differences between means. T-test against zero was used on MGD data. To determine statistical differences between regression lines, one-way ANCOVA was applied.

3.4 Results

Total dry weight at the final harvest was highest in AM and NM *F. psammophila* and AM *P. lanceolata*, while the smallest biomass was found in NM *H. pilosella*, *H. radicata* and *P. lanceolata* (Fig. 3.2a). Biomass of AM and NM plants was not significantly different in the grass species, whereas presence of AMF had a significant positive effect on biomass of all forb species ($p < 0.001$),. In presence of AMF, *H. pilosella* and *C. canescens* revealed noticeably similar total dry weights and root/shoot biomass allocation patterns.

H. pilosella showed the highest MGD (98.9%), followed by *H. radicata* (96.1%) and *P. lanceolata* (88.9%; Fig. 3.2b). In marked contrast to the forb species ($p < 0.001$), growth of the grasses was not affected by AMF, with *F. psammophila* and *C. canescens* exhibiting MGDs of 3.1% and -1.8%, respectively. See also Fig. 1.2.

Tissue P concentration in the grasses was not affected by presence of AMF (Fig. 3.2c). However, P concentration was lower ($p < 0.05$) in both AM and NM *C. canescens* than in AM and NM *F. psammophila*, respectively. Significant differences were found between AM and NM forbs: While AM forbs exhibited values of 2.15 (*P. lanceolata*), 2.77 (*H. pilosella*) and 2.41 (*H. radicata*) mg g dry weight^{-1}, the corresponding NM plants showed about 60-70% lower P concentrations ($p < 0.001$). However, P concentration in the AM forbs was similar to *F. psammophila*, while exceeding the values of *C. canescens*.

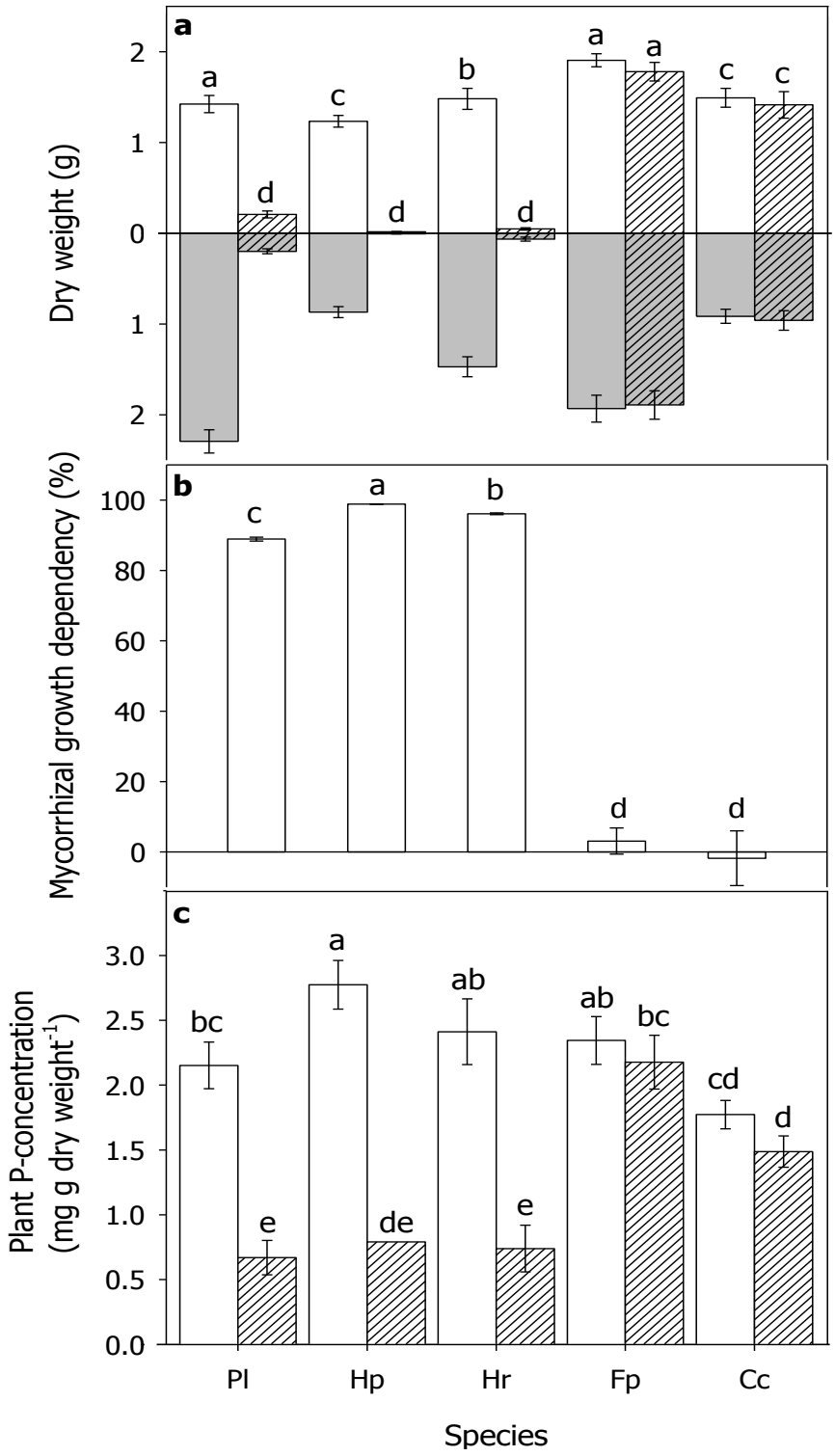

Fig. 3.2 Mean a) dry weight, b) mycorrhizal growth dependency and c) plant tissue phosphorus concentration of mycorrhizal (open bars) and non-mycorrhizal (hatched bars) plants of *Plantago lanceolata* (Pl), *Hieracium pilosella* (Hp), *Hypochaeris radicata* (Hr), *Festuca psammophila* (Fp) and *Corynephorus canescens* (Cc) after 20 weeks of growth (final harvest). Stacked columns (a) represent root (grey) and shoot (white) dry weight. Different letters indicate significant differences at p = 0.05 (ANOVA). Means ± SE, n = 8 (AM) and 6 (NM). Statistical results in plot a) refer to total dry weight.

While high fine root proportions were found for the AM grasses *C. canescens* (75%) and *F. psammophila* (74%), values were significantly lower ($p < 0.001$) in the AM forbs (37-49%; Fig. 3.3). Differences between fine root proportions of AM and NM plants were found in all three forb species: In absence of AMF, a significantly higher ($p < 0.001$) fine root proportion was detected in both *H. pilosella* and *H. radicata*, reaching values equal to the grasses, whereas this difference was less pronounced in *P. lanceolata* ($p < 0.01$). However, these effects are most likely a direct result of the generally much smaller size of the NM forbs and do not reflect adaptive strategies of NM mycorrhizal species. Fine root proportions of the grasses were not affected by inoculation.

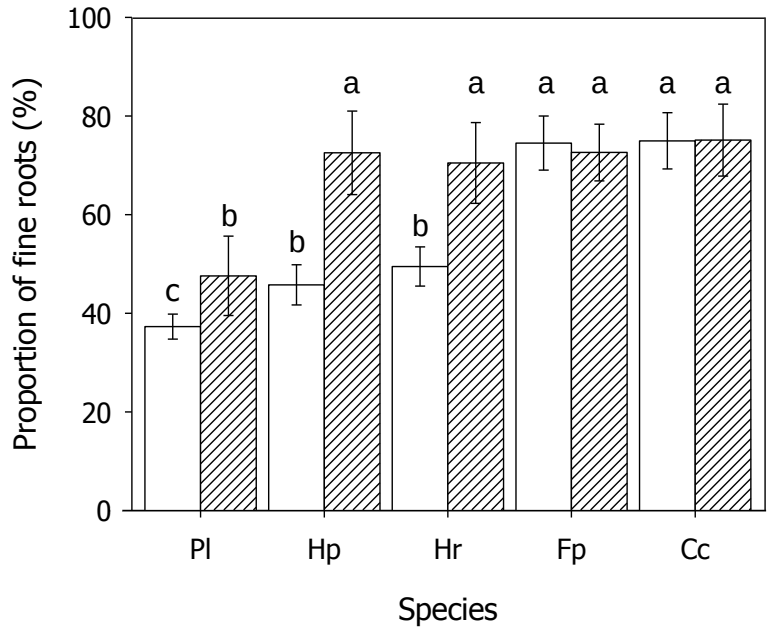

Fig. 3.3 Mean percentage of fine roots (diameter < 0.2 mm) in the extracted soil cores of mycorrhizal (open bars) and non-mycorrhizal (hatched bars) plants of *Plantago lanceolata* (Pl), *Hieracium pilosella* (Hp), *Hypochaeris radicata* (Hr), *Festuca psammophila* (Fp) and *Corynephorus canescens* (Cc) during the 3 ingrowth periods (sampling 2-4). Different letters indicate significant differences at $p = 0.05$ (ANOVA). Means for each individual across sampling 2-4 ± SE, n = 8 (AM) and 6 (NM).

Roots of *P. lanceolata*, *H. pilosella* and *H. radicata* were heavily colonized by AMF (94% in all three species), which was in marked contrast to the grasses, with *F. psammophila* and *C. canescens* exhibiting comparatively low colonization levels ($p < 0.001$) of 21% and 35%, respectively (Tab. 3.1). The abundance of vesicles was significantly different between forbs (21-23%) and grasses (0-2%) ($p < 0.001$). Abundance of arbuscules was variable within the two functional groups with the highest level in *H. pilosella* (23%), intermediate levels in *P. lanceolata* (14%), *H. radicata* and *C. canescens* (10%, respectively) and the lowest level in *F. psammophila* (2%). No colonization was observed in the uninoculated plants.

Tab. 3.1 Percentage of root length colonized by arbuscular mycorrhizal fungi and abundance of arbuscules and vesicles in the root samples of the fourth soil core extraction. Different letters indicate significant differences between species at $p = 0.05$ (ANOVA). Means ± SE, $n = 8$.

Species	Colonization (%)	Vesicles (%)	Arbuscules (%)
Plantago lanceolata	94 ± 1 a	21 ± 2 a	14 ± 5 a
Hieracium pilosella	94 ± 1 a	23 ± 2 a	23 ± 11 b
Hypochaeris radicata	94 ± 2 a	23 ± 2 a	10 ± 6 a
Festuca psammophila	21 ± 5 b	0 ± 0 b	2 ± 2 c
Corynephorus canescens	35 ± 5 c	2 ± 1 b	10 ± 6 a

The species-specific ratio between root and hyphal SA in the examined soil cores was constant across all four samplings, as indicated by coefficients of determination ranging from 0.89 to 0.99 (Fig. 3.4). All correlations were significant ($p < 0.05$) except for *F. psammophila* with an almost significant correlation ($p = 0.058$). The ratio between root and hyphal SA was markedly higher in grasses than in forbs ($p < 0.01$), as indicated by the different slopes of the regression lines (Fig. 3.4). *F. psammophila* produced a 7.4-fold higher root than hyphal SA, which was significantly higher as compared to *C. canescens* (2.7-fold higher root than hyphal SA; $p < 0.05$). In marked contrast, the relation between the two SA components was vice versa in the forbs, where hyphal surface largely exceeded the root surface, with *H. pilosella*, *P. lanceolata* and *H. radicata* producing a 3.45-, 3.23- and 2.04-fold higher hyphal than root SA, respectively.

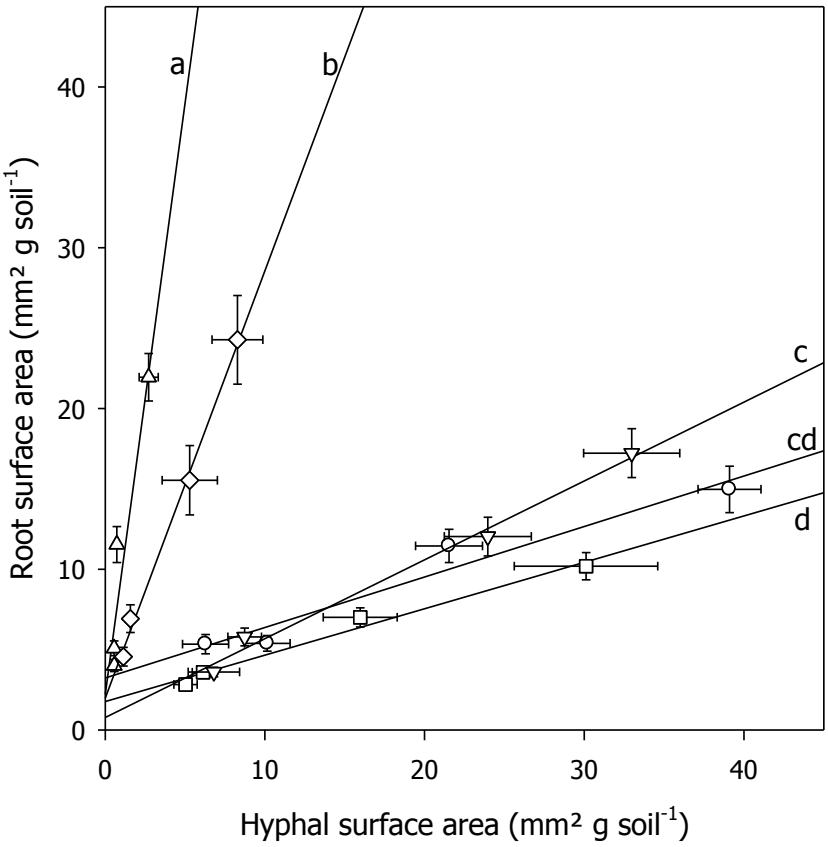

Fig. 3.4 Development of the relationship between hyphal and root surface area in the sampled soil cores of mycorrhizal *Plantago lanceolata* (circles, r^2=0.95; p = 0.026), *Hieracium pilosella* (squares, r^2=0.98; p = 0.009), *Hypochaeris radicata* (inverted triangles, r^2=0.99; p = 0.005), *Festuca psammophila* (triangles, r^2=0.89; p = 0.058) and *Corynephorus canescens* (diamonds, r^2=0.995; p = 0.002) across sampling 1-4. Different letters indicate significant differences at p = 0.05 (ANCOVA) between the species. Means ± SE, n = 8.

The AM forbs exhibited significantly ($p < 0.001$) higher total SA growth rates than the corresponding NM plants, whereas no differences were found between AM and NM grasses (Fig. 3.5). The total SA growth rate of AM *P. lanceolata* and *H. radicata* was significantly higher than that of both grasses ($p < 0.001$). The total SA growth rate of AM *H. pilosella* was significantly smaller than that of the other AM forbs ($p < 0.001$) , and only exceeded that of the grass *F. psammophila* ($p < 0.001$), while it was only marginally higher than that of AM *C. canescens*. Within all AM plants, *F. psammophila* exhibited the lowest total SA ($p < 0.001$). AM forbs and grasses had similar root SA growth rates, except *H. pilosella*, which exhibited a significantly lower value ($p < 0.05$) than all other species. The higher total SA growth rates in the AM forbs were due to significantly higher ($p < 0.05$) hyphal SA contributions as compared to the grasses.

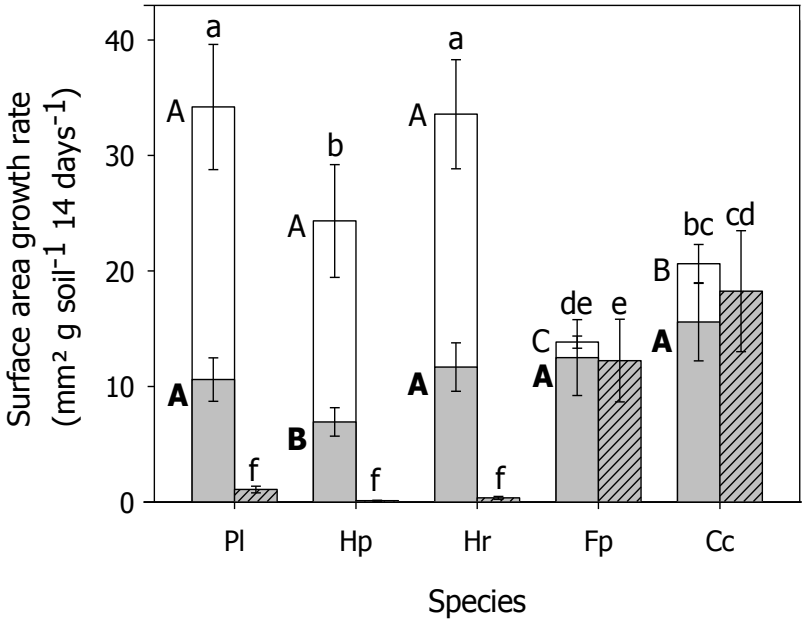

Fig. 3.5 Growth rates of root (grey bars) and hyphal (white bars) surface area of mycorrhizal (open bars) and non-mycorrhizal (hatched bars) *Plantago lanceolata* (Pl), *Hieracium pilosella* (Hp), *Hypochaeris radicata* (Hr), *Festuca psammophila* (Fp) and *Corynephorus canescens* (Cc). Different letters indicate significant differences at p = 0.05 (ANOVA) in total (lower case letters), root (bold upper case letters) and hyphal (upper case letters) surface area. Means across sampling 2-4 ± SE, n = 8 (AM) and 6 (NM).

Differences in P depletion rates between corresponding AM and NM treatments were neither found in the grasses *F. psammophila* and *C. canescens*, nor in the forb *H. pilosella* (Fig. 3.6, Tab. 3.2), while presence of AMF significantly increased P depletion in *P. lanceolata* and *H. radicata* with p = 0.005 and 0.017, respectively (Tab. 3.2). Temporal effects on P depletion where only significant in *P. lanceolata* (p = 0.0125), where also an interaction was found between the factors time and AMF (p = 0.0135).

Tab. 3.2 ANOVA results on P depletion rates of the five species (Fig. 3.6). Sums of squares (*SS)*, *F*- and p-values are given for the factors AMF presence (AMF) and sampling time (time) and for cross-interaction between the two factors (AMF X time). Boldface values are significant.

Species	Factor	df	*SS*	*F*	p
Plantago lanceolata	AMF	2	0.991	14.868	**0.0005**
	time	1	0.661	4.961	**0.0125**
	AMF X time	2	0.649	4.868	**0.0135**
Hieracium pilosella	AMF	2	0.121	1.914	0.1751
	time	1	0.132	1.041	0.3634
	AMF X time	2	0.220	1.736	0.1906
Hypochaeris radicata	AMF	2	0.777	11.492	**0.0017**
	time	1	0.431	3.193	0.0529
	AMF X time	2	0.340	2.519	0.0947
Festuca psammophila	AMF	2	0.122	2.885	0.0980
	time	1	0.133	1.568	0.2224
	AMF X time	2	0.047	0.558	0.5771
Corynephorus canescens	AMF	2	<0.001	0.003	0.9562
	time	1	0.071	0.322	0.7271
	AMF X time	2	0.271	1.229	0.3046

The 14-day P depletion rates were strongly positively correlated with the growth rates of the total SA (r^2 = 0.921; p = 0.010), with *F. psammophila* exhibiting the lowest value, *P. lanceolata* and *H. radicata* exhibiting the highest values and *H. pilosella* and *C. canescens*

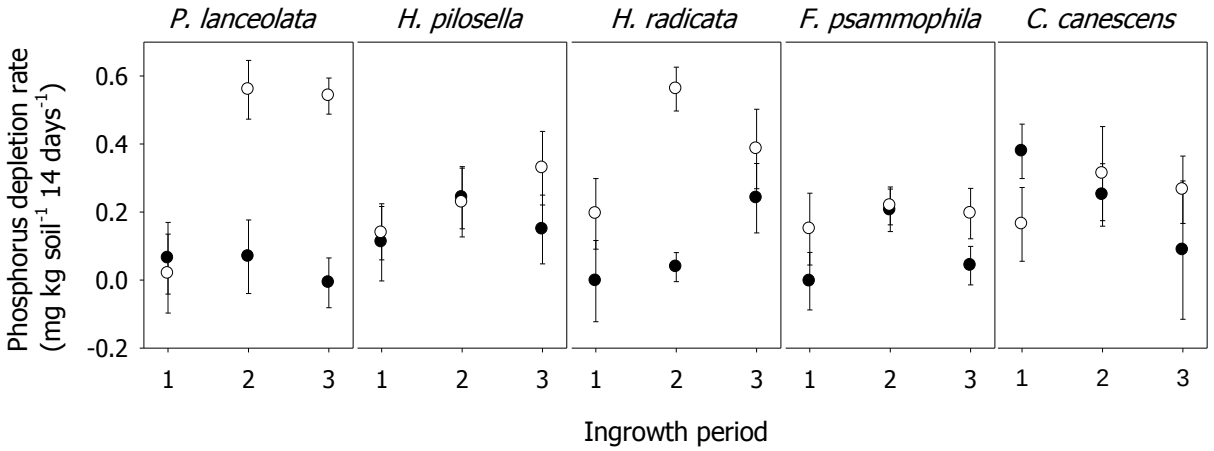

Fig. 3.6 Phosphorus (P) depletion rates of mycorrhizal (open circles) and non-mycorrhizal (closed circles) plants of *Plantago lanceolata*, *Hieracium pilosella*, *Hypochaeris radicata*, *Festuca psammophila* and *Corynephorus canescens* during the 3 ingrowth periods, expressed as P concentration decrease over 14 days. Means ± SE, n = 8 (AM) and 6 (NM). See Tab. 3.2 for ANOVA results.

having an intermediate position (Fig. 3.7c). The high total SA growth rates of *P. lanceolata* and *H. radicata*, and thus the high P depletion, were mainly driven by the major contributions of the hyphal SA (r^2 = 0.716; p = 0.071; Fig. 3.7b, see also Fig. 3.5), whereas root SA was not correlated with P depletion rates (r^2 = 0.004; p = 0.921; Fig. 3.7a).

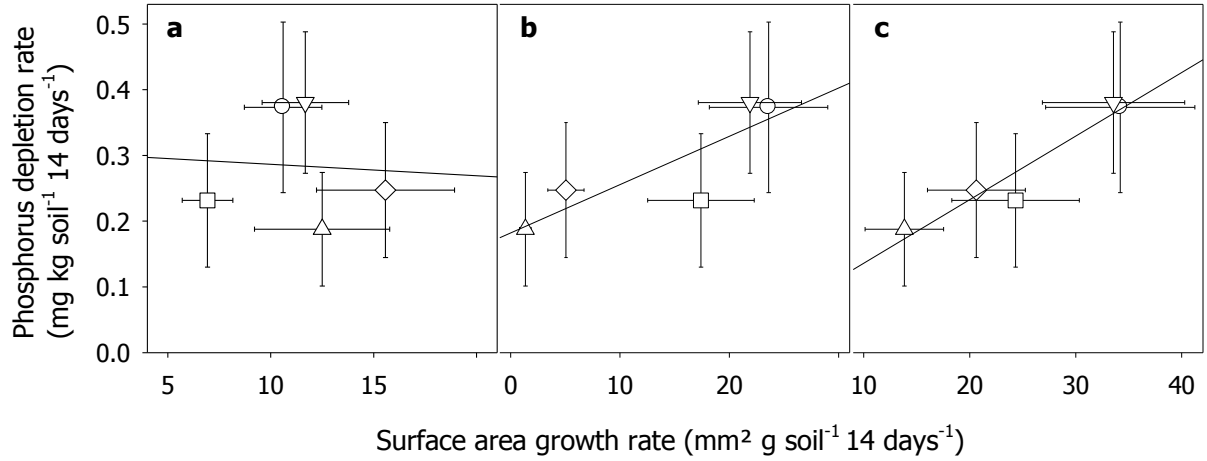

Fig. 3.7 Relation between mean phosphorus depletion rates and mean a) root (r^2 = 0.004, p = 0.921), b) hyphal (r^2 = 0.716, p = 0.071) and c) total (r^2 = 0.921, p = 0.010) surface area growth rates of AM *Plantago lanceolata* (circles), *Hieracium pilosella* (squares), *Hypochaeris radicata* (inverted triangles), *Festuca psammophila* (triangles) and *C. canescens* (diamonds). Means ± SE, n = 8.

3.5 Discussion

3.5.1 Contrasting dependencies on presence of AMF for P uptake and growth

A species is considered to be obligately mycorrhizal if it is always found to form mycorrhiza, while a species is considered to be facultatively mycorrhizal if it is reported to form mycorrhiza in one habitat but not in another (Wang & Qiu 2006; Smith & Read 2008). By this definition of the mycorrhizal status, the grasses *C. canescens* and *F. psammophila* have been classified as facultatively mycorrhizal, while the forbs *H. pilosella* and *P. lanceolata* have been described as obligately mycorrhizal (Wang & Qiu 2006). *H. radicata* is characterized as facultatively mycorrhizal by Wang & Qiu (2006), referring to Titus & del Moral (1998), who defined this species as 'facultatively mycotrophic'. However, the term 'mycotrophic' is exclusively related to nutritional dependency on mycorrhiza (Janos 2007) and thus may not refer to the mycorrhizal status. Since there is no literature information on natural occurrence of *H. radicata* without AM formation, we regard *H. radicata* here as obligately mycorrhizal, according to the definition of Wang & Qiu (2006). Although the mycorrhizal status alone may not resolve to which degree a plant is dependent on mycorrhiza for nutrition and growth, a generally lower dependency can be expected for facultatively than for obligately mycorrhizal species. We found growth to be strongly dependent on presence of AMF in the obligately mycorrhizal forbs (Figs. 3.2a, b) which supports our first hypothesis. The strong AMF-dependency of *H. pilosella* is in line with the findings of previous studies (Grime *et al.* 1987; van der Heijden *et al.* 1998). In contrast to our results, the only available study on growth response of *H. radicata* to presence of AMF (Titus & del Moral 1998), describes both, negative and slightly positive responses. There are several contradictory studies on the mycorrhizal responsiveness of *P. lanceolata* covering the entire range from negative over neutral to positive responses (Parádi *et al.* 2003; Ayres *et al.* 2006; Heinemeyer *et al.* 2006). However, the observed responsiveness of *P. lanceolata* was in no case as pronounced as in the present study. These discrepancies are explained by the fact that the responsiveness of a plant species to inoculation with AMF depends on factors such as fungal species identity, nutrient availability, light intensity and pot volume (Janos 2007; Hoeksema *et al.* 2010; Johnson 2010). In this study, the strong responsiveness of the three forbs to the presence of AMF was probably promoted by the relatively low soil P availability of approx. 2 mg kg^{-1}, chosen to simulate conditions of the early successional stages of temperate, open sand ecosystems (Süß *et al.* 2004). However, irrespective of the low P availability, the two studied grasses were clearly non-responsive to AMF inoculation with MGDs close to zero (Fig. 3.2b), thus AMF-independent in terms of growth. This has been described as a common trait among

the Pooideae (Reinhart *et al.* 2012). However, to our knowledge, this is the first report on the MGD of *F. psammophila* and *C. canescens*.

One of the major factors explaining AMF-dependency is P uptake via the mycorrhizal pathway, which may account for a large proportion of plant P acquisition (Li *et al.* 1991; Jakobsen *et al.* 1992; Cui & Caldwell 1996; Hetrick *et al.* 1996; Hartnett & Wilson 2002). The marked growth depression in the NM forbs was therefore most likely due to a restricted P acquisition of these highly AMF-dependent species. The obvious differences between tissue P concentrations of AM and NM forbs (Fig. 3.2c) confirm this assumption, indicating a significantly reduced P uptake in the NM forbs, which is in accordance with our hypothesis. This result underlines the high dependency of the forbs on the AM symbiosis, as an adequate P supply is essential for growth and a quantity of metabolic processes (Vance *et al.* 2003). In contrast, internal P concentrations of *C. canescens* and *F. psammophila* were not affected by the absence of AMF. However, the observed non-responsiveness of P uptake to presence of AMF in the grasses may not necessarily imply that the fungal partner is irrelevant for their P nutrition. It has been shown that, irrespective of 'visible' responsiveness, the AM P uptake pathway may substitute the direct P uptake pathway (via roots) by up to 100% (Smith *et al.* 2003). For example, Cui & Caldwell (1996) and Hetrick *et al.* (1996) showed major contributions of the AM P uptake pathway in the almost non-responsive grasses *Bromus inermis* and *Agropyron desertorum*. Therefore, it is possible that the AM P uptake pathway was also active in *C. canescens* and *F. psammophila*, but, in contrast to the investigated forbs, the grasses were capable to compensate any potential contribution of the AM P uptake pathway by the direct P uptake pathway in the absence of AMF.

The studied forbs obviously gained high nutritional benefits from the mycorrhizal association, indicating that this close mutualistic relationship is probably of major importance for their success in colonizing bare sand. On the other hand, the strong AMF-dependency might pose a disadvantage under conditions that are unfavorable for the performance of the fungal symbiont. In contrast, less mycotrophic pioneer species such as the studied grasses, would not suffer that much from absence or poor performance of the AMF.

3.5.2 Species-specific C allocation trade-off between roots and AMF

Besides the high dependencies on AMF for P uptake and growth, root morphological traits provide further support for the highly mycotrophic lifestyle of the studied forbs. In contrast to the grasses, which showed high proportions of fine roots (Fig. 3.3), the forbs exhibited a higher proportion of thicker roots, thus resulting in a comparatively lower specific root area.

Of course this is a rather unfavorable trait for the direct uptake of immobile nutrients, which is likely the reason for the high AMF-dependency (Baylis 1975). Microscopic measurements showed that the higher root diameter in the forbs results from a much thicker root cortex as compared to the grass roots (data not shown). There is anatomical evidence that roots originally evolved as habitats for mycorrhizal fungi and that the formation of a large cortex volume is a typical trait of obligately mycorrhizal plants, whereas non-mycorrhizal and facultatively mycorrhizal plants tend to have finer roots with thinner cortices (Baylis 1975; Hetrick *et al.* 1992; Schultz *et al.* 2001; Brundrett 2002; Seifert *et al.* 2009). The cortex, which only seems to have a purpose in mycorrhizal roots, is the largest organ in most primary roots (Brundrett 2002), and thus, its formation and maintenance are likely to require considerable amounts of C. Thus, high biomass allocation to the root cortex in the studied forbs may indicate an (additional) indirect C investment into the AMF. Accordingly, the roots of all three forb species were almost completely colonized by the fungus (Tab. 3.1). Since the formation of intraradical AMF organs has been linked to a high C supply by the host plant (Fitter 1991; Francis and Read 1995), the high abundance of these structures is a further indication for a high C transfer towards the AMF in the forbs. In contrast, intraradical fungal structures were rare in the grass roots (Tab. 3.1). Particularly the 10-fold higher abundance of vesicles indicates an excessive AMF-directed C allocation in the forbs as compared to the grasses, since AMF may store surplus C in form of lipids in these structures (Smith & Read 2008). These results support the hypothesized C allocation trade-off differences between forbs and grasses and confirm the proposed close mutualistic relationship between AMF and forbs under nutritional conditions prevalent in the early successional stages of open sand ecosystems.

In accordance with the differences between their intraradical AMF mycelia we found the two functional groups to differ in their specific C allocation between the production of root and hyphal SA, as the total absorptive SA was clearly dominated by hyphal growth in the forbs and by root growth in the grasses (Figs. 3.4, 3.5). Furthermore, the ratios between root and hyphal SA remained constant across the four soil core samplings in each species (Fig. 3.4), which makes age and size dependent effects on the belowground C allocation trade-off unlikely, but indicates that the measured ratios are strongly species-specific. These results clearly support our second hypothesis and are in line with previous suggestions on the trade-off between the production of extraradical AMF hyphae and fine roots (Miller *et al.* 1995; Rillig *et al.* 1999; Brundrett 2002), thus indicating contrasting foraging strategies among the studied sand ecosystem pioneer plant species.

3.5.3 Relevance of different C-allocation patterns for soil P depletion

Foraging via AM hyphae instead of roots may provide several advantages to the plant, e.g., lower costs in terms of belowground C investment (e.g., Harley 1989; Fitter 1991; Jakobsen *et al.* 1992; Schweiger *et al.* 1995), a higher phosphorus use efficiency (Koide *et al.* 2000) and a higher capacity of proliferation into small-scale nutrient patches due to the smaller size of hyphae as compared to roots (Hodge 2004). In this study, we focused on potential advantages provided by an AMF-mediated increase of the absorptive SA growth rate, as rapid proliferation into bare sand patches is one of the most important traits for the success of pioneer plants in the early successional stages of open sand ecosystems (Casper & Jackson 1997; Grime 2001; Jentsch & Beyschlag 2003). In accordance with our third hypothesis, the growth rates of the total absorptive SA were significantly higher in the AM forbs than in the grasses (with the exception of *H. pilosella*, which did not differ from *C. canescens*) (Fig. 3.5). As revealed by partitioning into root and hyphal contributions, the forbs' higher total SA growth rate was provided exclusively by the major contributions of hyphal growth, whereas root SA growth rates of AM forbs were similar (and in *H. pilosella* lower) as compared to the grasses. These findings indicate a potential advantage of the 'AMF strategy' over the 'fine root strategy' in terms of rapid growth into bare sand patches.

This advantage is reflected in significantly increased soil P depletion rates of the AM forbs *H. radicata* and *P. lanceolata* as compared to the grasses. Within the forbs, only AM *H. pilosella* did not increase P depletion, which is in line with a consistently smaller total absorptive SA observed in this species (Figs. 3.5, 3.6, Tab. 3.2). However, the observed differences between P depletion rates of the two functional groups were not reflected in tissue P concentrations (Fig. 3.2c), which could be explained by a potentially higher P depletion of the grasses at the pot level as compared to the measured P depletion within the soil cores. Even though P losses from the soil cores were found in each treatment (Fig. 3.6), it is unlikely that they were solely caused by root and hyphal depletion. This becomes obvious as e.g., P losses in NM *H. pilosella*, which grew almost no roots into the soil cores, were similar to those in *F. psammophila*, exhibiting an approx. 100-fold higher total SA growth (Fig. 3.5). However, although *F. psammophila* probably had a much higher P depletion rate than NM *H. pilosella*, we suggest that it was still relatively low (as compared to those of AM *P. lanceolata* and *H. radicata*) and that small P losses from the soil cores due to diffusion into the surrounding substrate might have restricted resolution of such low P depletion rates. Nevertheless, our data suggest that efficient P depletion within the soil cores was dependent on the respective total absorptive SA, because of the strong correlation between both

parameters (Fig. 3.7c). A comparison of the correlations between P depletion and the different SA components (i.e. root and hyphal SA, Fig. 3.7a, b) indicates that the large contributions of the hyphal SA to the total SA (see also Fig. 3.5) were the driving factor for P depletion. This is further supported by a direct comparison between the forb *H. pilosella* and the grass *C. canescens*. In the presence of AMF, both species exhibited an almost equal biomass and root/shoot-ratio (Fig. 3.2a) as well as an almost equal total SA growth rate (Fig. 3.5). Nevertheless, *H. pilosella* revealed significantly higher tissue P concentrations (Fig. 3.2c). Considering the fact that the total absorptive SA was made up to major proportions by hyphae in *H. pilosella* and by roots in *C. canescens* (Fig. 3.5), this might indicate an advantage of hyphal over root P uptake. However, the suggested higher P uptake of *H. pilosella* was not detectable in the 14-day depletion rates of the soil cores (Fig. 3.6). Probably, as mentioned above, the total SA growth rates of the forb *H. pilosella* and the grasses *F. psammophila* and *C. canescens* were too low to cause a resolvable decrease of the soil P concentration within 14 days, which is in contrast to *P. lanceolata* and *H. radicata* (Figs. 3.5, 3.6).

Our results indicate that foraging via mycorrhizal hyphae instead of roots may provide an advantage to pioneer plants in the early successional stages of open sand ecosystems, where P is scarce and mainly available from recently evolved bare sand patches. Therefore, a predominantly root-mediated foraging, such as in the studied grasses, might be a less effective strategy under such conditions. However, in contrast to nutrients acquired by roots, those taken up by an AMF mycelium are not exclusively available to one host plant, since mycorrhizal mycelia - and the nutrients, acquired by them - may be shared between competing plants (van der Heijden & Horton 2009). Besides the above-mentioned independency of fungal performance, this might be another reason for the successful establishment of less mycotrophic species such as *C. canescens* and *F. psammophila* in disturbed sand habitats. On the other hand, the rapid colonization of bare sand, as enabled by AMF-mediated foraging, might allow for the fast establishment of dominance stands, thus reducing interspecific competition, as e.g., frequently observed in *H. pilosella* (Bishop & Davy 1994).

Although nutrition and growth of the investigated forbs and grasses were clearly related to AMF and root morphology, we were not able to distinguish these factors from potential effects of different functional-group-related traits. Thus, facultatively mycorrhizal forb species and obligately mycotrophic grass species should be included in future studies.

3.6 Conclusions

Our study showed distinct foraging strategies of co-existing pioneer plants inhabiting open sand ecosystems, with the coarse-rooted forbs *P. lanceolata*, *H. pilosella* and *H. radicata* being highly dependent on the presence of AMF for P uptake and growth, while the fine-rooted grasses *F. psammophila* and *C. canescens* were revealed as AMF-independent under the given conditions. We found distinct species-specific root and hyphal contributions to the total absorptive SA with their relation to soil P depletion pointing towards an advantage of the 'AMF strategy' over the 'fine root strategy' in terms of rapid exploitation of P from bare sand patches. This trait may be particularly beneficial in frequently disturbed and nutrient-poor ecosystems. Thus, both species-specific AMF-dependencies as well as potential advantages of a predominantly AMF-mediated nutrient acquisition may have important implications for the performance of some pioneer plants in the early successional stages of temperate, open sand ecosystems.

3.7 Authors contributions

I designed the experiment and gave major contributions to the experimental work, data analysis and writing of this chapter. Further contributions were given by M. Friede, who took part in the experimental work and data analysis. S. Unger and W. Beyschlag assisted with data interpretation and writing. Further, I acknowledge contributions of E. Furlkröger, C. Schlüter and B. Teichner for support with plant cultivation and laboratory work.

This chapter has been published in:
Höpfner I, Friede M, Unger S, Beyschlag W. (2014) Potential advantages of highly mycotrophic foraging for the establishment of early successional pioneer plants on sand. Functional Plant Biology, http://dx.doi.org/10.1071/FP14097

CHAPTER 4

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Experiment 3

Intra- and interspecific seedling facilitation via common mycelial networks in *Hieracium pilosella* and *Plantago lanceolata*

4.1 Abstract

Belowground plant-plant interactions are highly relevant for successional processes in nutrient-deficient habitats such as the early successional stages of temperate European sand ecosystems. As predicted by the stress gradient hypothesis (SGH), positive plant-plant interactions, particularly between large plants and seedlings, may be important in these systems, as the occurring plants are faced with high levels of environmental stress. Considering that several plant species in these habitats are strictly dependent on AMF, facilitation of seedling establishment by large plants via CMN might play a role for success of these species. However, there is no information on the relevance of CMN-mediated seedling facilitation for interactions between early, stress-tolerant pioneer plants and subsequent plant species of higher competitive ability. Thus, we investigated CMN-mediated seedling facilitation between an obligately mycorrhizal stress-tolerator (*Hieracium pilosella*) and an obligately mycorrhizal competitor species (*Plantago lanceolata*) in a controlled pot experiment. Seedling growth was promoted by neighboring large plants providing CMNs, thus accelerating seedling root colonization. Net facilitative effects were highest when only *H. pilosella* large plants were present, whereas strong competitive pressure by *P. lanceolata* large plants overlaid beneficial CMN-effects. We found that positive effects by *H. pilosella* large plants were not restricted to seedlings of the same species, but also clearly promoted establishment of *P. lanceolata* seedlings, thus probably promoting the survival rate of any potential competitor. This suggests important implications for the vegetation dynamics in transition stages between pioneer plant communities and subsequent successional stages.

4.2 Introduction

Plant-plant interactions are of major importance for successional processes and plant species composition. Particularly resource competition between plants of the same or different species is of high ecological relevance as it may have important implications for community structure

(Tilman 1985). While competition for light gains rising importance during succession as a result of increasing vegetation density, earlier successional stages, where light is a less limiting resource, are dominated by belowground competition (Tilman 1988; Casper & Jackson 1997; Weiner *et al.* 1997). In this regard, European temperate, open sand ecosystems have gained scientific attention as belowground interactions between plants and their influence on community structure may be observed across different successional stages in these systems (Jentsch & Beyschlag 2003). For example, transitions between early successional stages dominated by stress-tolerating pioneer plants and subsequent stages dominated by plant species of higher competitive ability have been the topic of several studies on plant-plant interactions (e.g., Berendse & Elberse 1990; Weigelt *et al.* 2000; Fromm *et al.* 2002). Most of these studies focus on competitive interactions and their relevance for successional changes in community structure, but little is known about the role of facilitative plant-plant interactions for species success in these systems.

Here, facilitation is defined as a 'positive, non-trophic interaction that occurs between physiologically independent plants and is mediated through changes in the abiotic environment or through other organisms' (Brooker *et al.* 2008). Though often neglected in the past, facilitation between plants has gained considerable attention in the last two decades, with a quantity of recent studies on the underlying mechanisms (Holzapfel & Mahall 1999; Maestre *et al.* 2003) and on the relevance of facilitation in broader ecological contexts (Bruno *et al.* 2003; Tirado & Pugnaire 2003; Lortie *et al.* 2004; Kikvidze *et al.* 2005). Net facilitation is most frequently observed in stressful environments (e.g., Greenlee & Callaway 1996; Pugnaire & Luque 2001; Maestre *et al.* 2003; Gómez-Aparicio *et al.* 2004; Brooker *et al.* 2006; Callaway 2007) and several models predict that the intensity of facilitation is positively correlated with the stress level plants are exposed to (Bertness & Callaway 1994; Callaway & Walker 1997; Holmgren *et al.* 1997; Brooker & Callaghan 1998; Bruno *et al.* 2003; Brooker *et al.* 2008). This concept is commonly known as the stress-gradient hypothesis (SGH) and is usually related to the seminal paper of Bertness & Callaway (1994). The early successional stages of temperate, open sand ecosystems should provide favorable conditions for occurrence of facilitation as derived by the SGH, as these habitats reveal high levels of environmental stress such as high temperature, drought, nutrient deficiency and erosion (Jentsch & Beyschlag 2003).

Facilitation has been shown to be of particular importance for the establishment and survival of seedlings, as mortality is highest in this stage of a plant's life (Brooker *et al.* 2008). Neighboring large plants (here, we use 'large plants' instead of 'adult plants', as the latter term

might suggest that seedling and large plant automatically belong to the same species) may increase a seedling's survival probability by locally reducing the stress level or by promoting seedling growth, thus shortening the period of highest vulnerability (Brooker *et al.* 2008). Besides a quantity of reports on beneficial effects due to shading by large plants (e.g., Holmgren 2000; Lenz & Facelli 2003; Pagès *et al.* 2003), other mechanisms such as soil stabilization (Aerts *et al.* 2006; Yan *et al.* 2007), 'hydraulic lift' (Pate & Dawson 1999; Sekiya & Yano 2004) or improvements of the microbial community (Brooker *et al.* 2006), have also been reported. In particular, plant-plant facilitation as mediated by mutualistic mycorrhizal fungi has been the topic of several recent studies, many of which demonstrating that seedlings may be facilitated via CMNs, provided by neighboring large plants (see van der Heijden & Horton 2009). These networks of mycorrhizal hyphae interlink plant individuals of different species, age and size, and were found in all plant communities tested for their presence (Leake *et al.* 2004; van der Heijden & Horton 2009). The importance of hyphal networks for initiation of fast seedling root colonization by mycorrhizal fungi has been emphasized earlier (e.g., Grime *et al.* 1987; Friese & Allen 1991; Francis & Read 1995; van der Heijden 2004). CMNs might thus have the potential to reduce seedling mortality, as promotion of seedling root colonization may ensure a faster establishment, which, however, should be dependent on the reliance of a plant species on mycorrhiza for nutrition and growth. The generally high probability of facilitation in the early successional stages of temperate open sand ecosystems makes the occurrence of CMN-mediated seedling facilitation likely, particularly when considering that several plant species are highly dependent on AMF for nutrition under the prevalent conditions (Ch. 2, 3). Despite the potentially high importance of CMN-mediated facilitation for the survival rate of seedlings, the relevance of this mechanism has not yet been investigated in plants of the early successional stages of temperate European sand ecosystems.

Even though early successional stages are often dominated by non-mycorrhizal or facultatively mycorrhizal plants (Janos 1980; Miller 1987; Allen & Allen 1990), there are also obligately mycorrhizal plants, successfully colonizing bare sand, such as the forb *Hieracium pilosella* L. (Asteraceae), which is frequently dominant in pioneer plant communities (Jentsch & Beyschlag 2003). In a previous experiment, *H. pilosella* revealed as almost unable to grow on bare sand in absence of AMF, indicating mycorrhiza to be a key factor for its success in nutrient-deficient habitats (Ch. 2, 3). However, despite the generally high relevance of AMF in this species (Bishop & Davy 1994; van der Heijden *et al.* 1998), investigations on the relevance of intraspecific CMN-mediated seedling facilitation in *H. pilosella* are lacking. Furthermore, it is not known if potential facilitation by *H. pilosella* is restricted to seedlings of

the same species or if there is also interspecific facilitation of neighboring seedlings of other plant species.

We investigated large pant - seedling interactions between *H. pilosella* and *Plantago lanceolata* L. (Plantaginaceae), a less stress-tolerant, later successional species with high competitive strength (Ellenberg 1996). Both species are obligately mycorrhizal (Wang & Qiu 2006) and may co-occur in transition stages between *H. pilosella*-dominated and subsequent plant communities (Ellenberg 1996). After precultivation of large plants of both species either in intra- or interspecific combination in compartmented pots, seedlings of either *H. pilosella* or *P. lanceolata* were planted into a central compartment, only accessible for fungal hyphae but not for roots. Subsequent seedling establishment was compared to a control treatment without large plants to quantify the extent of facilitation. Measurements on seedling growth during the 'establishment period', as well as assessment of seedling biomass and fungal parameters at the end of the experiment, allowed for detecting species-specific differences in large plant - seedling interactions. We hypothesized that (1) *H. pilosella* large plants facilitate seedlings of both species via CMN and that (2) the extent of net facilitation is dependent on species identity of the neighboring large plants, with seedling establishment being generally stronger promoted by *H. pilosella* than by *P. lanceolata* large plants.

4.3 Material and Methods

4.3.1 Experimental setup

We performed a controlled pot experiment, investigating the effects of large plants on the growth performance of neighboring seedlings. Plants were cultivated in compartmented, rectangular pots (internal dimension 15 x 15 x 12 cm) with two outer large plant compartments (LPC), each comprising 40% of the pot volume, separated by a central seedling compartment (SC), comprising 20% of the pot volume (Fig. 4.1). Compartments were separated by nylon meshes (pore size 32 µm; Plastok Ltd., Birkenhead, UK), preventing direct root interactions between large plants and seedlings, but allowing fungal hyphae to pass. Each treatment pot contained two seedlings and two large plants of either *H. pilosella*, *P. lanceolata* or both, with one large plant individual grown in the center of each LPC and two seedling individuals of either *H. pilosella* or *P. lanceolata* symmetrically placed in the SC. Large plants were absent in the control treatment. The effects on the establishment of the two seedling species, as dependent on the large plant species combination, were compared to

the control treatment without large plants. We used three replicates (3 x 2 seedlings) per treatment, resulting in a total of 24 pots.

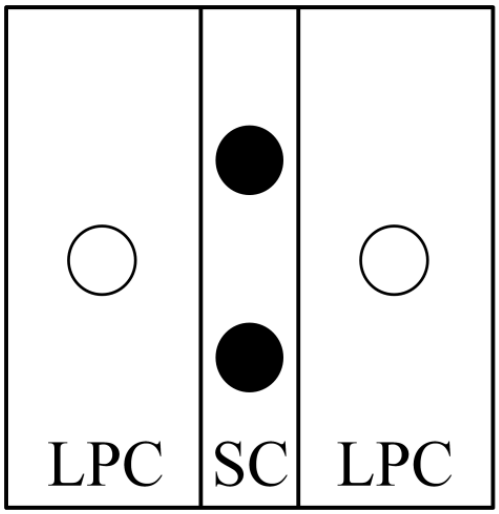

Fig. 4.1 Schematic representation of the compartmented-pot design, with two outer large plant compartments (LPC) and a central seedling compartment (SC), separated by 32µm nylon meshes (lines). Large plant pairs (open circles) of *Hieracium pilosella* and *Plantago lanceolata* were grown either in monoculture or mixture and combined with pairs of *H. pilosella* or *P. lanceolata* seedlings (closed circles), resulting in a total of eight treatments (n = 3).

4.3.2 Plant cultivation

The experiment was performed in a greenhouse at a light (photosynthetic photon flux density of approx. 300 µmol m^{-2} s^{-1}) / dark period of 14 h / 10 h, a temperature of 22 °C / 15 °C and a relative air humidity of approx. 60%. For precultivation of large plants, seeds of *H. pilosella* and *P. lanceolata* (Blauetikett-Bornträger GmbH, Offstein, Germany) were sown and started in boxes with autoclaved (120 °C for 1.5 h; FVA/A1, Fedegari, Switzerland) sand. Ten days after germination, the compartmented pots were filled with autoclaved sand and 'large plant seedlings' of equal size were selected and transferred to planting holes (diameter 1.8 cm; depth 7 cm) in the center of each LPC. Each plant was inoculated by filling the planting hole with a mixture of autoclaved sand (18 g) and an expanded-clay-inoculum (3.5 g; BioMyc GmbH, Brandenburg, Germany), containing infective units of the AMF *Rhizophagus intraradices* (N.C. SCHENCK & G.S. SMITH) C. WALKER & A. SCHÜßLER. Large plants were precultivated for 9 weeks, a period that revealed as sufficient for an effective mycorrhizal colonization of the used plant species under similar conditions in previous experiments. At the end of this period (64 days after planting; dap), two 10-day-old seedlings of the same species were planted into each SC, with a distance of 5 cm to both each other and the pot rims. All

seedlings were inoculated with AMF as described for the large plants, except for a smaller diameter of the planting holes (1.2 cm), thus minimizing disturbance of potentially established extraradical mycelia (ERM). Seedlings and large plants were then grown for another five weeks.

Once a week, a modified Hoagland fertilizer solution (1.5 mmol KNO_3, 0.5 mmol $Ca(NO_3)_2$, 0.25 mmol $(NH_4)_2SO_4$, 0.25 mmol KH_2PO_4, 0.5 mmol $MgSO_4$, 0.25 mmol KCl, 0.25 mmol $FeC_6H_5O_7$, 0.00625 µmol H_3BO_3, 0.0005 µmol $MnSO_4$, 0.0005 µmol $ZnSO_4$, 0.000125 µmol $CuSO_4$, 0.000125 µmol MoO_3 per liter; Hoagland & Arnon 1950) was applied to the LPCs. The volume of the applied fertilizer solution was adapted to the increasing demand of the growing plants, with 20, 25 and 30 ml per LPC from 0-14, 15-28 and 29-98 days after planting (dap), respectively. During precultivation of large plants (0-63 dap), the control pots were not fertilized to avoid an accumulation of nutrients. However, during seedling establishment (64-98 dap), all pots received the same amount of fertilizer. SCs were not fertilized during the whole experiment. To maintain a constant soil humidity of approx. 6 %, all pots were individually watered with deionized water to nominal weight twice a week. Once a week, pot positions were randomized to rule out location effects. Shading effects on the seedlings by large plant leaves did not occur.

4.3.3 Determination of net large plant effects on seedling growth

Seedling total leaf area was assessed twice a week during the establishment period to detect potential large plant effects on seedling growth dynamics. As we were not able to measure the leaf area of living seedlings directly, this parameter was calculated on base of the length of the longest leaf, the total count of leaves and the species-specific ratio between leaf length and area. Based on the observation that leaf lengths within an individual seedling were distributed almost homogeneously between zero and the maximum leaf length, we calculated the total leaf area of seedlings by

$$\mathrm{LA}_{\mathrm{seedling}} = \sum_{k=1}^{n} k \cdot \frac{\text{max length}}{n} \cdot \mathrm{R} \qquad \text{Eq. 4.1}$$

, with $\mathrm{LA}_{\mathrm{seedling}}$ = total seedling leaf area; max length = length of the longest leaf; n = number of leaves and R = species-specific ratio between leaf area and leaf length, as calculated by measuring and averaging this ratio for 10 representative leaves of each species subsequent to the final harvest, with $R_{\textit{H. pilosella}} = 5$ (cotyledon = 5) $R_{\textit{P. lanceolata}} = 2.8$ (cotyledon = 1.6).

At the end of the experiment (98 dap), all plants were harvested and divided into root and shoot biomass. Total leaf area was measured (Delta T Devices Ltd., Digital Image

Analysis System Version 1.12, Cambridge, UK) and root fresh weight was determined. Large plant and seedling shoots as well as large plant root material was dried at 70 °C for 5 d and weighed. Measurement of seedling root dry weight was not possible, as the comparatively small seedling root samples were entirely needed for quantification of mycorrhizal colonization, which required fresh samples. Thus, seedling root dry weight was calculated on base of the species-specific dry weight to fresh weight ratios of the large plants (0.099 ± 0.002 and 0.104 ± 0.007 for *H. pilosella* and *P. lanceolata*, respectively).

4.3.4 Fungal measurements

During final harvest, substrate was separately sampled from all LPCs and SCs and dried at 40 °C. Extraradical hyphae were quantified, using an aqueous extraction and membrane filter technique adapted from Jakobsen *et al.* (1992). Twenty g of dried substrate were suspended in a solution of 100 ml deionized water and 12 ml sodium hexametaphosphate solution (35 g l^{-1}) and vigorously shaken for 30 s. After 1 h, the suspension was transferred to a 40 µm sieve. The material on the sieve was rinsed gently with deionized water to remove clay particles and transferred to a 250 ml Erlenmeyer flask which was subsequently filled with 200 ml deionized water. The flask was shaken thoroughly for 10 s to flotate the hyphae. After 60 s, an aliquot of 20 ml was taken from a defined height of the supernatant and drawn through a 25 mm membrane filter (0.45 µm pore size). Fungal material on the filter was specifically stained with a Trypan Blue solution (5:1 = (2:1:2 = lactic acid : glycerin: H_2O) : Trypan Blue (0.4%, Sigma-Aldrich Chemie GmbH, Germany)) for 5 min. After rinsing with deionized water, the filter was transferred to a microscope slide and hyphal density expressed as hyphal length per soil dry weight was determined according to Miller *et al.* (1995) at x 250 magnification.

For quantification of intraradical mycorrhizal structures the fresh root samples of the seedlings were bleached in 10 % KOH at 90°C for 10 min, rinsed with deionized water and stained with an ink-acetic-acid solution (1:1:8 = ink : 10% acetic-acid : H_2O) at 90°C for 15 min, followed by a final, intense rinsing with deionized water (Phillips & Hayman 1970). The root fragments were then transferred to microscope slides and the percentage of root length colonized by AMF was estimated at x 250 magnification using a modified intersect method (McGonigle *et al.* 1990), scoring a minimum of 100 intersections per sample for the presence of hyphae, vesicles and arbuscules.

4.3.5 Statistical analyses

Statistical analyses were performed using Statistica 6.0 (StatSoft Inc., Tulsa, USA). Since pairs of seedlings, large plants and LPC-mycelia within the same pot could not be treated as independent samples, data were averaged across each pot, resulting in three final replicates per treatment. Calculation of means from the three replicates included standard error propagation. In the LP species mix, LP and LPC-mycelium values within each species (n = 3) were used as true replicates. Analysis of differences between LPs was performed across data of the two seedling treatments (n = 6). Data were tested for normal distribution (Kolmogoroff-Smirnov test) and homogeneity of variances (Brown-Forsythe test) before analysis of variance.

Repeated-measures one-way ANOVA was performed on leaf area growth data, testing for effects of LP treatment and time, and for a cross-interaction between the two factors. One-way ANOVA was performed on data of seedling and LP dry weight and root/shoot ratio, on colonization level, arbuscule and vesicle abundances as well as hyphal densities, testing for the effect of LP treatments within each seedling treatment. LP treatment effects on LP dry weight and root/shoot ratio were compared among the two LP species. Differences in seedling colonization between control and LP treatments were analyzed by pairwise comparison (Student's *t*-test), as the control treatment was excluded from the ANOVA. Two-way ANOVA was performed on large plant biomass and root/shoot ratio data, testing for effects of seedling species and LP treatment, and for a cross-interaction between the two factors. When ANOVA revealed significant main effects, Fisher's LSD post-hoc test was applied.

4.4 Results

Seedling leaf area growth during the 'establishment period' was significantly affected by large plant treatments in both seedling species (Fig. 4.2) and a highly significant interaction between the factors LP treatment and time (Tab. 4.1). Shoot growth was on average stronger in *P. lanceolata* than in *H. pilosella* seedlings, as indicated by a higher *F*-value for the factor 'time' (Tab. 4.1). In both seedling species, growth was lowest in the control treatment, whereas presence of large plants induced a positive growth response, which was highest in *H. pilosella* LP monocultures (Fig. 4.2). Significant differences ($p < 0.05$) between control seedlings and those in LP treatments occurred first at 16 dpi. In *H. pilosella* seedlings, the lowest and an intermediate growth response were found in *P. lanceolata* LP monocultures and LP species mix, respectively (Fig. 4.2a). Differences within the three LP treatments were less

pronounced in *P. lanceolata* seedlings (Fig. 4.2b). Influence of LP treatments on leaf area development was stronger in *H. pilosella* seedlings than in *P. lanceolata* seedlings, as indicated by higher *F*- and lower p-values for this factor in *H. pilosella* (Tab. 4.1).

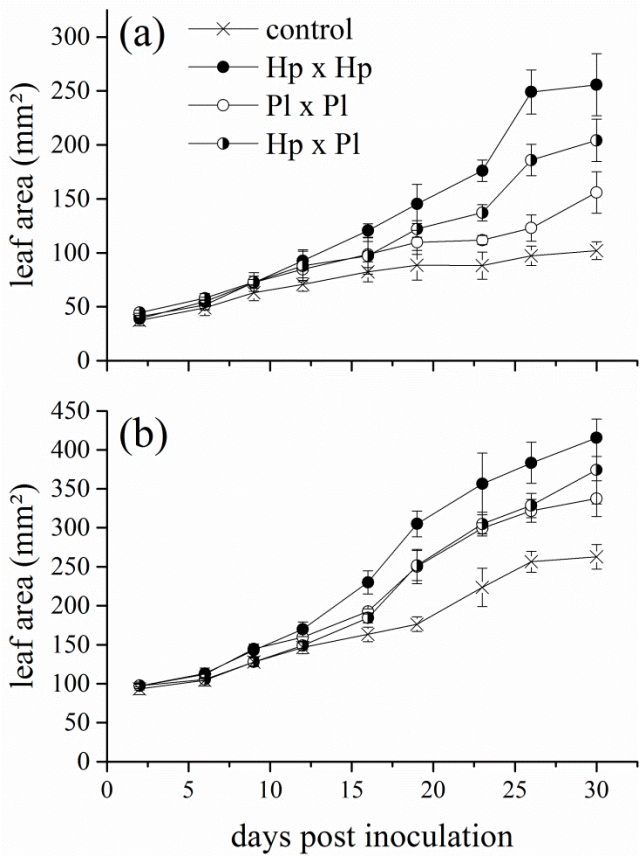

Fig. 4.2 Leaf area growth in (a) *Hieracium pilosella* and (b) *Plantago lanceolata* seedlings during the 'establishment period', dependent on large plant treatment (control: no large plant; Hp x Hp: *H. pilosella* monoculture; Pl x Pl: *P. lanceolata* monoculture; Hp x Pl: species mix). Means ± SE, n = 3. Note different scaling.

Tab. 4.1 Results of repeated-measures one-way ANOVA on the leaf area growth of seedlings during the establishment period. Sums of squares (*SS)*, *F*- and p-values are given for the factors large plant (LP) treatment and time and for cross-interaction between the two factors. Boldface values indicate significant effects.

Seedling Species	Factor	df	*SS*	*F*	p
Hieracium pilosella	LP treatment	3	50663	8.353	**0.0076**
	time	8	217969	94.510	< **0.0001**
	LP treatment X time	24	51030	7.375	< **0.0001**
Plantago lanceolata	LP treatment	3	72669	7.511	**0.0103**
	time	8	865199	393.972	< **0.0001**
	LP treatment X time	24	50580	7.677	< **0.0001**

Total dry weight of *P. lanceolata* seedlings was approx. 3-fold higher than that of *H. pilosella* seedlings in all LP treatments (Fig. 4.3). Seedlings of both *H. pilosella* and *P. lanceolata* revealed the lowest biomass in the control treatment, whereas it was increased in presence of large plants ($p < 0.05$ and $p < 0.001$, respectively). These positive growth responses were highest in *H. pilosella* LP monocultures, lowest in *P. lanceolata* LP monocultures, and intermediate in the LP mix. This response pattern was independent of the seedling species. However, in *H. pilosella* seedlings, differences to the control were only significant in *H. pilosella* LP monoculture (+ 170%; $p < 0.01$), while the LP species mix only induced an almost significant increase (+ 90%; $p = 0.079$). In contrast, in *P. lanceolata* seedlings, biomass was significantly increased in both, *H. pilosella* LP monoculture (+ 98%; $p < 0.001$) and LP species mix (+ 45%; $p = 0.012$). Biomass differences between controls and *P. lanceolata* LP monoculture was neither significant for *H. pilosella* (+ 53%; $p = 0.272$) nor *P. lanceolata* (+ 24%; $p = 0.109$) seedlings.

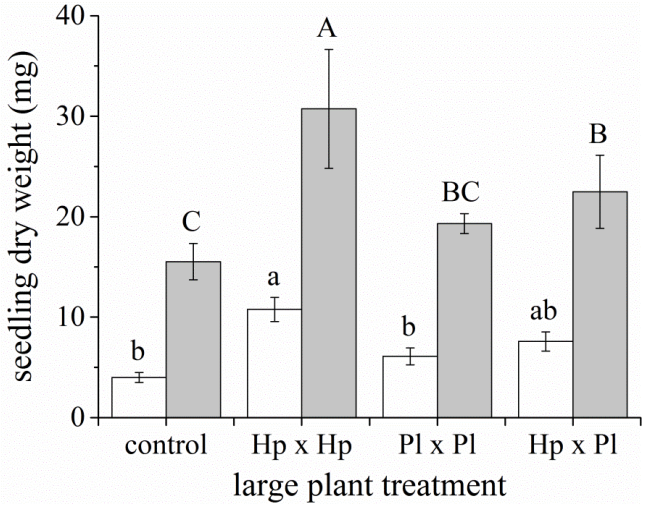

Fig. 4.3 Total dry weight of *Hieracium pilosella* (white bars) and *Plantago lanceolata* (grey bars) seedlings, dependent on large plant (LP) treatment (control: no large plant; Hp x Hp: *H. pilosella* monoculture; Pl x Pl: *P. lanceolata* monoculture; Hp x Pl: species mix). Different minor and capital letters indicate significant differences between LP treatments within *H. pilosella* and *P. lanceolata* seedlings, at $p = 0.05$ (ANOVA), respectively. Means ± SE, n=3.

Root/shoot ratios were higher in *P. lanceolata* seedlings than in *H. pilosella* seedlings (Tab. 4.2). While LP treatments had no effect on root/shoot ratio in *H. pilosella* seedlings, *P. lanceolata* seedlings exhibited an increased root/shoot ratio in the control treatment, whereas root/shoot ratio was significantly lower ($p < 0.05$) in *P. lanceolata* LP monoculture and LP species mix, and marginally lower ($p = 0.077$) in *H. pilosella* LP monocultures.

Tab. 4.2 Seedling root/shoot ratio (by dry weight), dependent on large plant (LP) treatment (control: no large plant; Hp x Hp: *H. pilosella* monoculture; Pl x Pl: *P. lanceolata* monoculture; Hp x Pl: species mix). Different letters indicate significant differences between LP treatments within each seedling species at $p = 0.05$ (ANOVA). Means ± SE, n = 3.

Seedling species	LP treatment	root/shoot ratio
Hieracium pilosella	control	0.46 ± 0.09 a
	Hp x Hp	0.42 ± 0.01 a
	Pl x Pl	0.46 ± 0.09 a
	Hp x Pl	0.36 ± 0.06 a
Plantago lanceolata	control	0.76 ± 0.07 a
	Hp x Hp	0.64 ± 0.23 ab
	Pl x Pl	0.60 ± 0.05 b
	Hp x Pl	0.55 ± 0.11 b

Density of extraradical hyphae in both LPCs and SCs was significantly lower ($p < 0.05$) in the control treatment than in the LP treatments (Fig. 4.4a, b), with this difference being on average stronger in *H. pilosella* than in *P. lanceolata* seedlings. However, significant differences within the three LP treatments were lacking, except for hyphal density in the LPCs of *P. lanceolata* LP monocultures with neighboring *H. pilosella* seedlings, which was significantly lower ($p < 0.05$) than in *H. pilosella* LP monocultures (Fig. 4.4a). Further, there was an overall tendency towards higher hyphal densities in SCs as compared to the corresponding LPCs (Fig. 4.4a, b).

A similar pattern was observed in the frequency of intraradical fungal structures, with total colonization as well as vesicle and arbuscule abundance being significantly lower ($p < 0.0001$) in the controls than in the LP treatments, whereas significant differences within the three LP treatments were lacking in each of the three measured parameters (Fig. 4.4c, d).

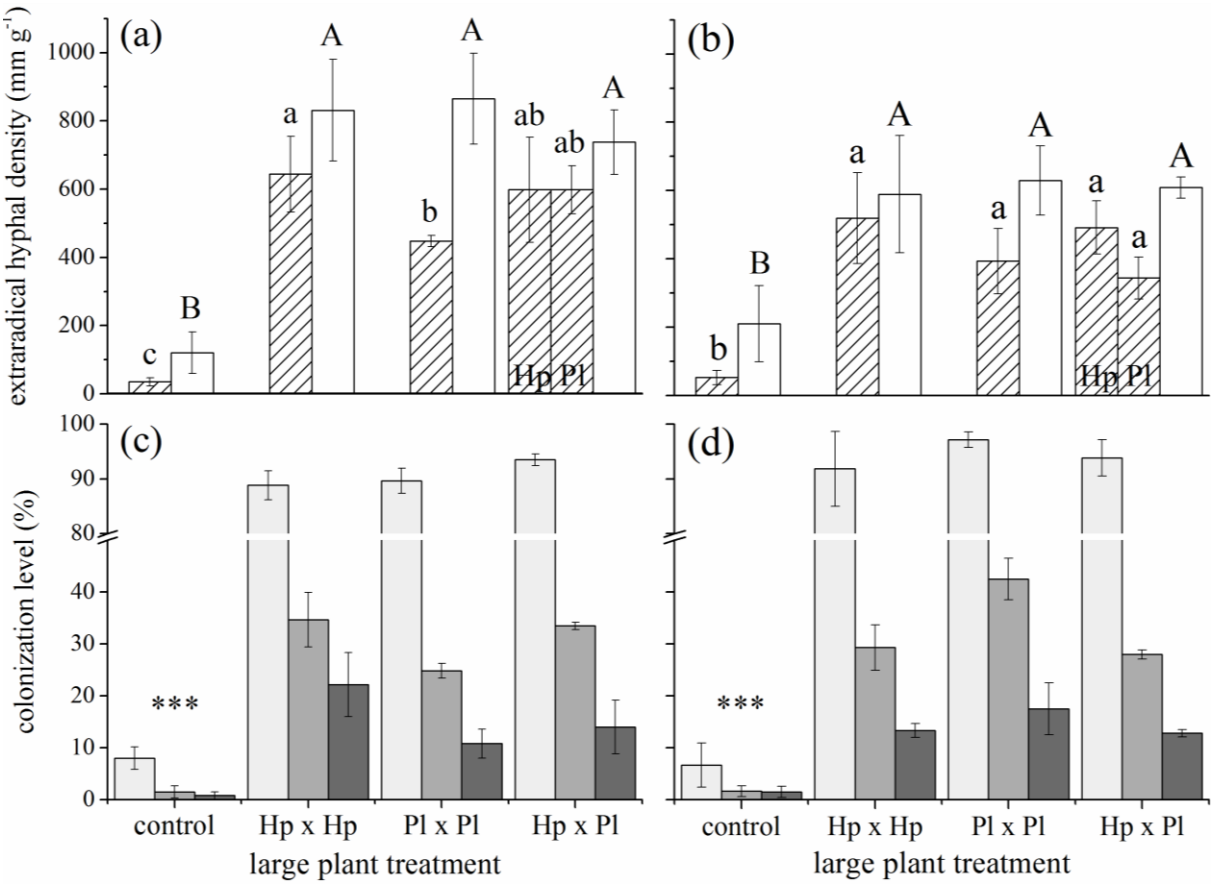

Fig. 4.4: Density of extraradical hyphae in (a) *Hieracium pilosella* and (b) *Plantago lanceolata* seedling compartments (white bars) and neighboring large plant (LP) compartments (hatched bars, subdivided into *H. pilosella* (Hp) and *P. lanceolata* (Pl) LP compartment in the HP x Pl - treatment), and mycorrhizal root colonization in (c) *H. pilosella* and (d) *P. lanceolata* seedlings, with total colonization (light grey bars), arbuscule abundance (grey bars) and vesicle abundance (dark grey bars), dependent on LP treatment (control: absence of large plants; Hp x Hp: *H. pilosella* monoculture; Pl x Pl: *P. lanceolata* monoculture; Hp x Pl: species mix). Different minor and capital letters in (a) and (b) indicate significant differences at $p = 0.05$ (ANOVA) between hyphal densities within LP and seedling compartments, respectively. Asterisks in (c) and (d) represent a highly significant ($p < 0.0001$) difference between control and large plant treatments in all three parameters. Means ± SE, n = 3.

Tab. 4.3 Results of two-way-ANOVA on large plant biomass and root/shoot ratio. Sums of squares (*SS)*, *F*- and p-values are given for the factors seedling species and large plant (LP) treatment and for cross-interaction between the two factors.

LP species	Parameter	Factor	df	*SS*	*F*	p
Hieracium pilosella	biomass	seedling species	1	0.0073	0.299	0.6000
		LP treatment	1	0.0015	0.061	0.8107
		sdlg. spec. X LP trmt.	1	0.0097	0.398	0.5467
	root/shoot ratio	seedling species	1	0.0010	0.303	0.5968
		LP treatment	1	0.0079	2.438	0.1570
		sdlg. spec. X LP trmt.	1	0.0061	1.884	0.2072
Plantago lanceolata	biomass	seedling species	1	0.0276	2.240	0.1728
		LP treatment	1	< 0.001	0.003	0.9560
		sdlg. spec. X LP trmt.	1	0.0383	3.108	0.1159
	root/shoot ratio	seedling species	1	0.0226	0.308	0.5940
		LP treatment	1	< 0.001	0.002	0.9682
		sdlg. spec. X LP trmt.	1	< 0.001	0.006	0.9410

Tab. 4.4 Total dry weight and root/shoot ratio of large plants (LP), dependent on LP treatment (Hp x Hp: *H. pilosella* monoculture; Pl x PL: *P. lanceolata* monoculture; Hp x Pl: species mix). Different letters indicate significant differences among LP species and treatments at p = 0.05 (ANOVA, Fisher's LSD post-hoc test). Means ± SE, n = 6.

LP species	LP treatment	total dry weight (g)	root/shoot ratio
Hieracium pilosella	Hp x Hp	0.46 ± 0.04 b	0.65 ± 0.03 b
	Hp x Pl	0.43 ± 0.08 b	0.60 ± 0.02 b
Plantago lanceolata	Pl x Pl	0.67 ± 0.05 a	0.82 ± 0.06 a
	Hp x Pl	0.67 ± 0.06 a	0.81 ± 0.14 a

Large plant biomass and root/shoot ratio was neither affected by the different LP treatments (intra- vs. interspecific combination) nor by the species identity of neighboring seedlings (Tab. 4.3). Thus, for a comparison of large plant biomass and root/shoot ratio as dependent on LP species and LP treatment, values were averaged within each LP treatment across *H. pilosella* and *P. lanceolata* seedling treatments (Tab. 4.4). *P. lanceolata* LPs

revealed significantly higher ($p < 0.05$) total dry weight and root/shoot ratio than *H. pilosella* LPs. However, the two parameters were in no case different between intra- and interspecific LP combinations (Tab. 4.4).

4.5 Discussion

4.5.1 CMN-mediated facilitation of seedlings by *H. pilosella* large plants

Based on the results on seedling and fungal growth, our first hypothesis, that *H. pilosella* large plants facilitate growth of both, *H. pilosella* and *P. lanceolata* seedlings via CMN, can be accepted. A generally positive effect of *H. pilosella* large plants on seedling establishment was indicated by a higher final seedling biomass in presence of *H. pilosella* large plants as compared to the control treatment (Fig. 4.3). These beneficial effects were also reflected in faster seedling leaf area development (Fig. 4.2, Tab. 4.1). Thus, our results indicate a clearly enhanced seedling establishment due to presence of *H. pilosella* large plants. Most likely, the observed positive growth response was CMN-mediated, as indicated by the markedly higher densities of extraradical hyphae in presence than in absence of large plants (Fig. 4.4a, b), that were accompanied by considerably increased seedling colonization levels (Fig. 4.4c, d), pointing towards a potentially high relevance of AMF for seedling growth. Since both, seedlings with and without neighboring large plants had been inoculated with AMF in the same way, enhanced seedling colonization must have been due to connection to extraradical mycelia provided by large plants. Thus, the enhanced colonization levels in presence of large plants indicate that large plants and seedlings were interlinked by a CMN. In previous experiments under similar conditions, the two investigated species revealed as highly dependent on AMF, with their growth performance being considerably increased by inoculation with AMF, whereas non-inoculated seedlings were almost unable to grow (Ch. 2, 3). Thus, the low colonization level in the control seedlings is a likely explanation for their low growth performance. Since fertilizer application to LPCs during precultivation was kept at a relatively low level, which had been proofed to limit growth of the investigated plant species under similar conditions (Ch. 2), an excess of nutrients in the LP treatment is an unlikely explanation for higher seedling growth in the LP treatments.

For the first time, we showed that CMN-mediated facilitation may play an important role for seedling establishment in pioneer species of the early successional stages of temperate open sand ecosystems. Our results are in line with other studies, demonstrating net CMN-mediated seedling facilitation by neighboring large plants (e.g., Grime *et al.* 1987; Francis &

Read 1995; Marler *et al.* 1999; Carey *et al.* 2004; van der Heijden 2004). In most cases, positive CMN-effects can been attributed to accelerated seedling root colonization and early access to CMN-nutrients (Read *et al.* 1985; Friese & Allen 1991; Read 1992; Olsson *et al.* 2002; Leake *et al.* 2004; Simard & Durall 2004; Hart & Reader 2005), which probably was also the main reason for seedling growth promotion in our experiment, as indicated by high colonization levels in presence of large plants. Moreover, as revealed by seedling growth curves, facilitative effects manifested already two weeks after seedling transplantation (Fig. 4.2), indicating that the present CMNs were highly efficient in colonizing seedling roots, thus providing early access to CMN-nutrients. This mechanism may be of particular importance for seedlings of highly mycotrophic species such as *H. pilosella* and *P. lanceolata* (Ch. 2, 3), especially when establishing under nutrient-deficient conditions as prevalent in the early successional stages of temperate, open sand ecosystems. Our results suggest that CMN-mediated facilitation by large plants might be of major importance for establishment of *H. pilosella* seedlings on bare sand, thus potentially increasing the survival rate and consequently species success. In this regard, CMN-mediated facilitation not only of the establishment of seedlings but also of vegetative offspring may play an important role in *H. pilosella*, as it frequently forms dense, clonal dominance stands (Bishop & Davy 1994).Thus, it is likely that the establishment of ramets and seedlings would benefit similarly from a CMN provided by a neighboring large (mother) plant. In accordance with our first hypothesis, facilitation by *H. pilosella* large plants was not restricted to seedlings of the same species, but also clearly promoted growth of *P. lanceolata* seedlings. Interspecific CMN-mediated seedling facilitation has already been observed earlier and (Moora & Zobel 2010) and should be quite common between arbuscular mycorrhizal plants due to the low host-specificity of AMF (Smith & Read 2008). Similar as in *H. pilosella* seedlings, the accelerated establishment can be expected to reduce seedling mortality in *P. lanceolata*. Thus, under stressful conditions, success of *P. lanceolata* may be promoted by presence of *H. pilosella* via provision of CMNs, enabling rapid mycorrhizal colonization. The observed interspecific seedling facilitation might have important implications for the success of the weak competitor *H. pilosella* (Bartelheimer et al. 2006) on the community level, as it may promote the introduction and establishment of potentially strong competitor species.

4.5.2 Effects of *P. lanceolata* large plants on CMN-mediated seedling facilitation

Based on the results on seedling shoot growth and biomass, we can accept the second hypothesis, that the extent of net facilitation is dependent on the species identity of

neighboring large plants. As hypothesized, promotion of seedling establishment was strongest in *H. pilosella* LP monocultures, while no significant net facilitation occurred in *P. lanceolata* monocultures, and intermediate facilitation was found in the LP species mix. One possible explanation for these differences is that growth of the extraradical mycelium and thus its inoculation potential may differ between plant individuals of different species, age and size (Brundrett *et al.* 1985; Rosewarne *et al.* 1997; van der Heijden & Horton 2009). However, even though extraradical mycorrhizal mycelia produced by *P. lanceolata* LPs were on average marginally smaller than those produced by *H. pilosella* (Fig. 4.4a, b), the observed differences in net facilitation can probably not be explained by different inoculation potentials of the respective CMNs, as seedling colonization levels were similarly high in all three LP treatments (Fig. 4.4c, d). There are several studies reporting no net facilitation or even negative CMN-effects on seedlings (Francis & Read 1995; Moora & Zobel 1996; Jakobsen 2004; Nakano-Hylander & Olsson 2007; Janouskova *et al.* 2011; Janos *et al.* 2013; Merrild *et al.* 2013). Furthermore, net positive large plant effects on seedling growth are generally only temporary, caused by the fact that as the initially facilitated seedling grows, resource demand and thus competition with large plants increases (Beltrán *et al.* 2012). Most likely, this was also the reason for the distinct seedling growth responses in this study. We suggest that positive effects were reduced as a result of competitive pressure by large plants, with the strongest and lowest reduction of net facilitation in *P. lanceolata* and *H. pilosella* LP monocultures, respectively. When only one *P. lanceolata* LP individual was present (LP species mix), total competitive pressure was intermediate. This result is in line with other reports on a generally high competitive strength of *P. lanceolata* (Berendse 1983; Ellenberg 1996; Berendse & Möller 2009), whereas *H. pilosella* has been revealed as a weak competitor in direct competition (Bartelheimer *et al.* 2006). However, since direct interactions between the roots of large plants and seedlings were prevented, competition for nutrients was only possible via diffusion across compartments or via CMN. It was suggested that competition for CMN-nutrients is probably driven by C supply of individual host plants to the CMN (Bücking & Shachar-Hill 2005; Lekberg *et al.* 2010; Kiers *et al.* 2011; Fellbaum *et al.* 2012; Merrild *et al.* 2013). In this regard, Wermerijewizc & Janos (2013) recently hypothesized that CMN-mediated competition might be size-symmetric - similar to root competition (Weiner 1986, Weiner *et al.* 2001) - with the highest amounts of CMN-nutrients transferred to the largest host plants, supplying most C to the network. In this regard, it has to be considered that not only the size of a plant but also its mycotrophy level should be an important determinant for the amount of C allocated to the AMF (Brundrett 2002). However, since *H. pilosella* and

P. lanceolata revealed similar mycotrophy levels and relative AMF-directed C allocation in previous experiments (Ch. 2, 3), we suggest that plant size was the main factor for species-specific differences in C allocation to the CMN. Stronger CMN-mediated competitive pressure on seedlings by *P. lanceolata* than by *H. pilosella* is thus quite likely, since *P. lanceolata* large plants revealed an on average ~ 50% higher biomass than *H. pilosella* large plants (Tab. 4.4). Additionally, *P. lanceolata* LPs exhibited proportionally larger root systems (Tab. 4.4), thus providing a larger plant-fungal interface for C-transfer to the CMN than *H. pilosella*. Therefore, CMN-mediated competitive pressure is a likely explanation for the reduced net seedling facilitation in presence of *P. lanceolata* large plants.

Although the high nutrient demand of *P. lanceolata* large plants obviously was sufficient to reduce CMN-mediated seedling facilitation, competition between large plant individuals was lacking, as revealed by LP dry weights and root/shoot ratios that did not differ between intra- and interspecific combinations (Tab. 4.4). Similarly, large plants were not affected by the different seedling treatments (Tab. 4.3). Thus, the performance of and the effects by each large plant species were comparable among all treatments.

P. lanceolata has been demonstrated to clearly dominate competitive interactions with *H. pilosella* plants of the same age in a previous experiment under similar conditions (Ch. 2). However, the present study suggests that the severe competitive pressure by *P. lanceolata* might not only limit growth and reproduction of already established *H. pilosella* plants, but that once introduced into a *H. pilosella*-dominated plant community, a strong competitor species might constrain establishment of *H. pilosella* seedlings as competitive effects might overlay beneficial CMN-effects. Establishment of *P. lanceolata* in a *H. pilosella*-dominated pioneer plant community might thus have serious negative consequences for the performance of the weak competitor *H. pilosella* on the community level. However, since replacement by (mycorrhizal) competitors of subsequent successional stages is likely and might even be promoted by their facilitation via CMN, preventing foreign seedlings from germinating in *H. pilosella*-stands might be a successful strategy. Indeed, *H. pilosella* often forms dense dominance stands by vegetative growth (Bishop & Davy 1994), thus potentially impeding nearby germination of seedlings as a method of reducing interspecific competition.

4.6 Conclusions

Our results suggest that facilitation of seedlings by large plants via CMN might be an important trait for the success of highly mycotrophic plants like *H. pilosella* in stressful environments such as during the colonization of bare sand. The principal mechanism

underlying the beneficial large plant effects was an accelerated mycorrhizal colonization of seedling roots. However, although seedling root colonization was equally promoted in presence of *P. lanceolata* large plants, simultaneous strong competitive effects overlaid beneficial effects. In contrast, a combination of low competitive pressure and provision of an effective CMN as by *H. pilosella* large plants seems to be highly beneficial to neighboring seedlings. In this regard, the observation that CMN-mediated facilitation is not restricted to seedlings of the same species but may also considerably promote the seedling establishment of stronger competitors such as *P. lanceolata*, might have important implications for the transition dynamics between *H. pilosella*-dominated and subsequent successional stages.

4.7 Authors contributions

I designed the experiment and gave major contributions to the experimental work, data analysis and writing of this chapter. Further contributions were given by M. Hefner, who took part in the experimental work and data analysis. S. Unger and W. Beyschlag assisted with data interpretation and writing. Further, I acknowledge contributions of E. Furlkröger and B. Teichner for support with plant cultivation and laboratory work.

CHAPTER 5

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Experiment 4

Investigating the mechanisms of CMN-mediated seedling facilitation: Relevance of seedling CMN-costs, large plant species identity and competitive effects for the net outcome of facilitation

5.1 Abstract

Seedling facilitation by neighboring large plants via CMNs occurs in the majority of plant communities and may be particularly relevant in stressful habitats. Positive CMN-effects are due to accelerated mycorrhizal colonization of seedling roots, thus providing an advantage over seedlings in absence of CMNs as a result of enhanced nutrition and lower C-costs for development of intraradical fungal structures. Nevertheless, the underlying mechanisms and the interplay between positive and negative CMN-effects are only poorly understood. In this regard, the net facilitation potential of CMNs has revealed as quite variable, which may be explained by differentially strong competitive pressure by neighboring large plants, overlaying positive effects to different extents. However, it is not known, to what extent the differences in net facilitation might be driven by large plant C-supply to a CMN, potentially differing with plant size and mycotrophy level, and thus differentially affecting seedling CMN-costs. In the present study we used a novel compartmented pot approach with selective elimination of host plant CMN-connections in combination with pulse chase labeling and nutritional analyses. We tried to disentangle, if certain 'key species' maintain a CMN by disproportionately high C supply, thereby reducing seedling CMN-costs. Although our results indicate that CMN-growth might be particularly promoted by productive, highly mycotrophic plants, we found that seedling CMN-costs were not reduced but increased by the CMN-connection of large plants, with this increase being independent of species identity and mycotrophic degree. In contrast, the extent of the CMN seemed to be the driving factor for increased seedling CMN-costs. We conclude that, besides competition for CMN-nutrients with neighboring large plants, increased CMN-costs may represent another negative CMN-effect, counteracting positive CMN-effects by accelerated inoculation. However, our results suggest that compensation of the positive 'inoculation effect' was rather due to negative effects by CMN-mediated competition for N than by increased seedling C-costs to the CMN. Moreover, our study suggests that, irrespective of limited seedling growth due to strong

competition for CMN-N, provision of a CMN might be essential for enabling P uptake in seedlings of highly mycotrophic species.

5.2 Introduction

About 80% of all land plants are associated with AMF, which transfer soil nutrients to their host plants and in return receive assimilated carbon (Smith & Read 2008). Some plant species are highly mycotrophic, i.e., that they are almost completely dependent on AMF for nutrition and growth under the conditions in their natural habitats (Janos 1980), whereas co-occurring plant species may be less or non-mycotrophic due to evolution of alternative foraging strategies (Baylis 1975). For example, high levels of mycotrophy have been shown in some sand pioneer plants of the early successional stages of temperate open sand ecosystems (Ch. 2, 3). Even though highly mycotrophic plants may benefit from AMF throughout their whole life-cycle, we propose that under stressful conditions as during growth on bare sand (Jentsch & Beyschlag 2003), the mutualistic relationship might be particularly important during seedling establishment, the most vulnerable stage of a plant's life cycle (Larcher 2003). Thus, limitation of seedling growth due to nutrient deficiency may be a major problem, as it prolongs the period of highest vulnerability. In this regard, it has to be considered that, in contrast to seedlings of less or non-mycotrophic species, nutrient acquisition in highly mycotrophic seedlings is dependent on appropriate root colonization by AMF.

One possibility by which a (mycotrophic) seedling may achieve the required mycorrhizal root colonization, is infection of roots by hyphae emerging from germinated fungal spores in the soil (Parniske 2008). Here, initial hyphal growth (towards roots) is completely reliant on the C reserves in the spore, allowing only for comparatively slow colonization (Bonfante & Perotto 1995). Accordingly, the improvement of nutrient acquisition and growth of the seedling is relatively slow. However, in most plant communities the soil is already interweaved by extensive CMNs, which are maintained by established plants and may even interlink individuals of different plant species, age or size (Harley 1991; Leake *et al.* 2004; van der Heijden & Horton 2009). In most natural systems, seedlings typically germinate next to established plants and their roots are in most cases efficiently colonized by already present CMNs, which should clearly accelerate the process of root colonization (Read *et al.* 1985; Read 1992; Olsson *et al.* 2002; Leake *et al.* 2004; Simard & Durall 2004). It has been suggested that the main positive CMN-effect on seedling growth is in fact the result of accelerated seedling colonization ('inoculation effect'), comprising at least two beneficial mechanisms: (1) CMN-colonized seedlings gain an advantage over seedlings without CMN-

support due to an earlier achievement of an adequate P-nutrition (Leake *et al.* 2004) and (2) there is a C-related advantage, since C-costs for root colonization are reduced, thus enabling the seedling to invest the 'saved' C into growth (e.g., Brundrett *et al.* 1985; Grime *et al.* 1987; Rosewarne *et al.* 1997). Although seedlings of mycorrhizal plant species should generally benefit from a CMN, as they are more or less dependent on adequate mycorrhizal root colonization for sufficient nutrient uptake (Janos 2007; see also Ch. 3), net CMN-effects are quite variable, ranging from positive over neutral to negative seedling growth responses (van der Heijden & Horton 2009). This is supported by a preliminary experiment of ourselves, where we found clear differences in the net facilitative CMN-effect on *Plantago lanceolata* and *Hieracium pilosella* seedlings, dependent on the species identity of neighboring large plants (see Ch. 4). Several studies showed negative CMN-effects on seedling performance caused by competition with large plants for CMN-nutrients (e.g., Eissenstat & Newman 1990; Kytoviita *et al.* 2003; Merrild *et al.* 2013; Fellbaum *et al.* 2014). On the other hand, it is not clear if differences in the net facilitation potential of a CMN may also be related to different large plant C-contributions and subsequently varying C-costs for the seedling to the established CMN. As outlined by van der Heijden & Horton (2009), an important question in understanding the mechanisms underlying CMN-mediated plant-plant interactions is, whether there are certain plant species maintaining a CMN by disproportionately high C supply, whereas others contribute less C.

Both, competition with large plants (equal to Ch. 4, here we use 'large plants' instead of 'adult plants', as the latter term might suggest that seedling and large plant automatically belong to the same species) for soil nutrients and seedling C-costs to a CMN are likely to contribute to the outcome of seedling facilitation, which in the end depends on species identity of the CMN-hosts. However, neither disentangling the importance of either mechanism in CMN-mediated seedling facilitation nor relating its outcome to host species identity in a CMN has never been achieved before. In this regard, the use of stable isotopes promises a quantitative separation of actual seedlings carbon investment into biomass production vs. CMN maintenance costs. In particular, pulse chase labeling of plants with $^{13}CO_2$, has been proven most suitable for tracing both transfer and fate of recent carbon, thereby identifying its consumers in the plant-soil continuum until it is released as respiratory CO_2 (e.g., Leake *et al.* 2006; Bradford *et al.* 2007; Ostle *et al.* 2007; Hoegberg *et al.* 2008). The present study aims to quantitatively analyze CMN-costs to seedlings and competition effects on seedlings sustained by a CMN as dependent on the mycotrophy level of different host species by the use of $^{13}CO_2$ pulse tracing in a novel experimental design (see Figures 5.1

and 5.2) enabling species-specific exclusion of host plants from a CMN in combination with the quantification of nutrient dynamics between seedlings and host plants. Thus a separation of the net CMN-effect on seedling growth into the (positive) 'inoculation' effect and the effects by retaining large plant CMN-connections was possible. Moreover, the novel approach allowed for subdividing the effects by retaining large plant CMN-connections into (negative) competitive effects and (presumably positive) effects by large plant C-supply to the CMN, and enabled relating these effects to large plant mycotrophy levels and size.

We set up an experiment enabling quantification of growth, CMN-costs, as well as N and P nutrition of *P. lanceolata* seedlings, dependent on species-specific exclusion of large plants from a CMN. We proposed that specific large plant C-supply to the CMN is dependent on the mycotrophy level of the respective species and compared between exclusion of the highly mycotrophic forbs *Hieracium pilosella* and *P. lanceolata* and the low mycotrophic grasses *Corynephorus canescens* and *Festuca psammophila*. We used pots with four outer compartments, each of which containing one individual of the four large plant species, separated from a central CMN-compartment by meshes permeable for mycorrhizal hyphae but not for roots (Fig. 5.1). This design enabled comparison of the effects by (1) retaining the CMN-connections of all four species, (2) specifically disrupting the CMN-connection of one or (3) all four large plants (Fig. 5.2). An additional treatment, in which hyphal access to the CMN-compartment by large plants was prevented during the entire experiment allowed for distinguishing between large plant effects by CMN provision ('inoculation effect', see above) and recent large plant effects via active CMN-connections. We analyzed CMN growth and seedling growth, root colonization and nutrient acquisition, as well as nutrient depletion from the CMN-compartment. For assessing the seedling CMN-costs, seedlings were $^{13}CO_2$-pulse-labeled prior to harvest and seedling C-allocation to the CMN was estimated by quantification of ^{13}C-label in both plant and extraradical fungal tissues. Further, a novel technique for assessing ^{13}C-label partitions in soil respiration enabled analysis of seedling-C transfer via hyphal connections within the CMN.

We hypothesized that (1) the CMN is to major extent maintained by the highly mycotrophic forbs *H. pilosella* and *P. lanceolata* and that therefore, (2) seedling CMN-costs are highest, when *H. pilosella* or *P. lanceolata* is excluded from the CMN, whereas exclusion of the less mycotrophic grasses *C. canescens* and *F. psammophila* would have minor effects on seedling CMN-costs. We expected net facilitative effects on seedling growth to be affected by both seedling CMN-costs and competition with large plants for CMN-nutrients. Since we assumed similarly high facilitative effects on seedling C-costs by *H. pilosella* and

P. lanceolata, but a higher competitive pressure by *P. lanceolata*, we hypothesized that (3) net facilitation is most reduced by exclusion of *H. pilosella*, to a lesser extent by exclusion of *P. lanceolata* and least by exclusion of the less mycotrophic grasses.

5.3 Material and Methods

5.3.1 Growth conditions and experimental set-up

Seeds of *Hieracium pilosella* L., *Plantago lanceolata* L., *Corynephorus canescens* (L.) P. BEAUV. and *Festuca psammophila* (HACK. EX ČELAK.) FRITSCH (Blauetikett-Bornträger GmbH, Offstein, Germany; *Botanical Garden* of the Westfälische Wilhelms-Universität *Münster*, Germany) were sown and started in boxes with sterilized (120°C for 1.5 h) sand. Two weeks after germination 50 individuals per species were transplanted to small trays (100 ml) with serilized sand. During transplantation, seedling roots were inoculated using 20 g of an inoculum-sand-mixture (*Rhizophagus intraradices*, (N.C. SCHENCK & G.S. SMITH) C. WALKER & A. SCHÜßLER, INOQ GmbH, Schnega, Germany). Plants were cultivated in a growth chamber at a light (photosynthetic photon flux density of approx. 430 µmol m^{-2} s^{-1}) / dark period of 14 h / 10 h, a temperature of 22 °C / 15 °C and a relative air humidity of 65 %. Twice a week, 5 ml of a modified Hoagland fertilizer solution (1.5 mmol KNO_3, 0.5 mmol $Ca(NO_3)_2$, 0.25 mmol $(NH_4)_2SO_4$, 0.25 mmol $(NH_4)_2HPO_4$, 0.5 mmol $MgSO_4$, 0.25 mmol KCl, 0.25 mmol $FeC_6H_5O_7$, 0.00625 µmol H_3BO_3, 0.0005 µmol $MnSO_4$, 0.0005 µmol $ZnSO_4$, 0.000125 µmol $CuSO_4$, 0.000125 µmol MoO_3; per liter; Hoagland & Arnon 1950) was applied. Plants were watered regularly with deionized water, thereby maintaining a constant soil moisture of approx. 6 %.

Thirty dap, 168 plants (42 of each species; 'large plants') were transferred to 42 compartmented pots (16 x 16 x 12 cm) with one individual of each species being planted to one of the four outer LP-compartments of each pot (Fig. 5.1, 5.2). LP-compartments were separated from a central CMN- compartment by PVC barriers with circular windows (diameter 8.6 cm), that were covered with a nylon mesh (pore size 32 µm, Plastok Ltd., Birkenhead, UK), permeable for extraradical hyphae but not for roots. The sand in the CMN-compartment had been muffled (650 °C for 6 h) previous to the experiment, in order to completely remove all organic compounds, which has revealed important in previous experiments because organic matter entangled in hyphae may falsify hyphal quantification (see below). However, normal sterilized sand was used for the LP-compartments. During the following 30 d ('establishment period') 5 ml of fertilizer solution (as described above) was applied to each

LP-compartment twice a week. Growth chamber settings were the same as during precultivation and were not changed throughout the whole experiment. In six of the 42 pots, a thin steel sheet was slid along all four mesh barriers once a day, in order to sever passing extraradical hyphae, thus preventing the establishment of a mycorrhizal mycelium in the CMN-compartment ('no CMN'-treatment; Fig. 5.2). All other treatments remained undisturbed during the establishment period.

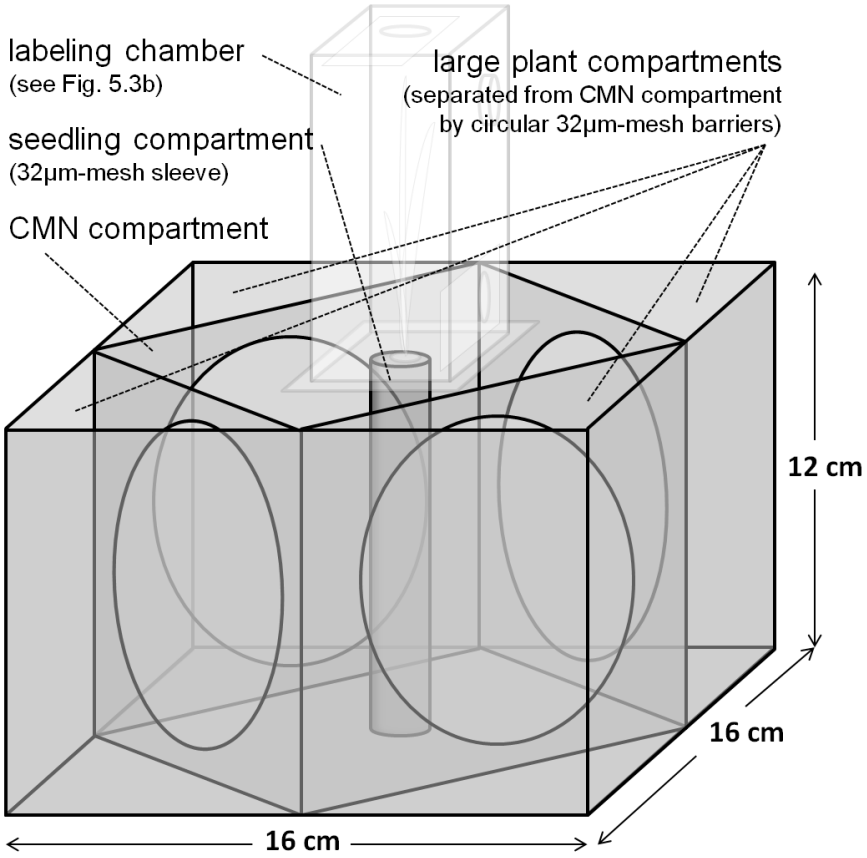

Fig. 5.1 Schematic of the compartmented-pot design.

As described in the following, the 'target' *P. lanceolata* seedlings were not directly planted into the CMN-compartment, but were restricted to a separate seedling-compartment, intending a spatial separation of seedling roots from CMN-hyphae. During the establishment period of the large plants, the 'target' seedlings were raised in sterilized sand. Two weeks after germination, 42 seedlings of equal size were selected, transplanted to cylindrical nylon mesh sleeves (pore size 32 µm; length 11cm; diameter 2 cm) and inoculated with 20 g of the above-mentioned sand-inoculum-mixture. Thus prepared, the seedlings were kept in sterilized sand for another eight days with subsequent transplantation to the CMN-compartments at the end of the establishment period of the large plants (60 dap). Cylindrical holes (diameter 2.5 cm, depth 10.5 cm) were drilled into the centre of each CMN-compartment, using a cork borer.

The extracted substrate was sampled for determination of hyphal density, N and P content (see section 5.3.7). The 3-week-old, mesh-wrapped *P. lanceolata* seedlings were then transplanted to the holes in the CMN-compartment. The small gaps (~ 3 mm) between mesh sleeve and CMN-substrate were filled up with sieved, muffled (see above) sand and wetted. The mesh sleeves ended slightly above the soil niveau in order to prevent root growth into the CMN-compartment. Each seedling received a plastic plate surrounding the stem base (base plate) in preparation to fix the labeling chamber on it (see Fig. 5.3b, 5.4 and description in section 5.3.2).

Depending on the treatment, hyphal connections of large plants to the CMN-compartment remained either intact (treatment 'full CMN'; Fig. 5.2) or were specifically disrupted daily in either only one ('specific exclusion' treatments: 'CMN -*Hp*', 'CMN -*Pl*', 'CMN -*Cc*' and 'CMN -*Fp*') or all (treatments 'CMN -all' and 'no CMN') of the four species during the following 27 d ('facilitation period'). Fertilization was the same as in the establish-

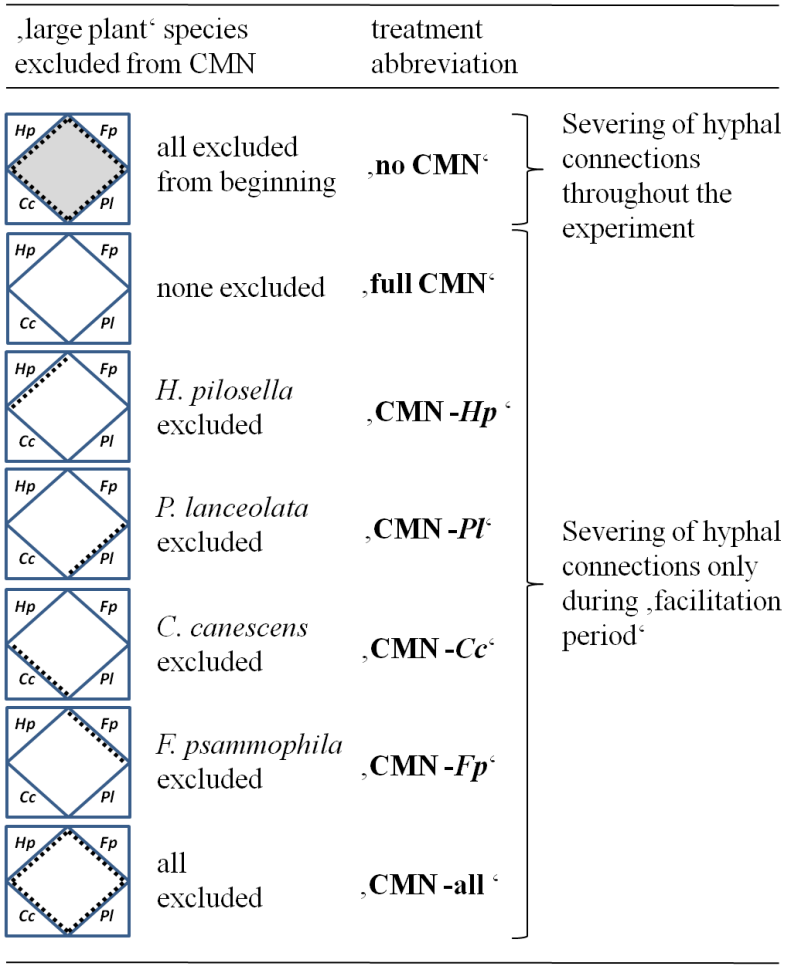

Fig 5.2 Schematic of the seven treatments. Dotted lines in pots indicate interruption of hyphal connections during the establishment period. Six replicates of each treatment were used.

ment period. In addition, twice a week 5 ml of fertilizer was applied to four symmetrically distributed spots (i.e., 20 ml in total) in the CMN-compartment, located directly between seedling and LP-compartments. Once a week, the pot positions were randomized to rule out edge and location effects. Aboveground-competition between the plants did not occur.

Comparison between 'no CMN' and 'CMN -all' allowed for assessment of the 'inoculation effect', whereas comparison between 'CMN -all' and 'full CMN' enabled determination of recent large plant effects via active CMN-connections. Comparison between 'full CMN' and the 'specific exclusion' treatments allowed for detecting the specific contributions of the different large plant species to the main ('full CMN') effects.

5.3.2 $^{13}CO_2$ pulse-labeling

Seedlings were $^{13}CO_2$-pulse-labeled seven and two days before final harvest (80 and 85 dap, respectively) using the setup depicted in Fig. 5.3a-c. Double labeling was necessary in order to obtain a higher resolution of incorporated label in the analyzed plant and fungal tissues. After sealing the gap between seedling trunk and base plate with terostat (Terostat IX, Teroson, Henkel AG & Co. KGaA, Munich, Germany) the seedling was enclosed into a transparent polyethylene chamber (100 cm³, Fig. 5.3b, 5.4). The gap between chamber and base plate was also sealed with terostat. The system was proved gas-tight in pre-tests, thus ruling out label losses through leaks. Using a gas-tight syringe (SGE International, Victoria,

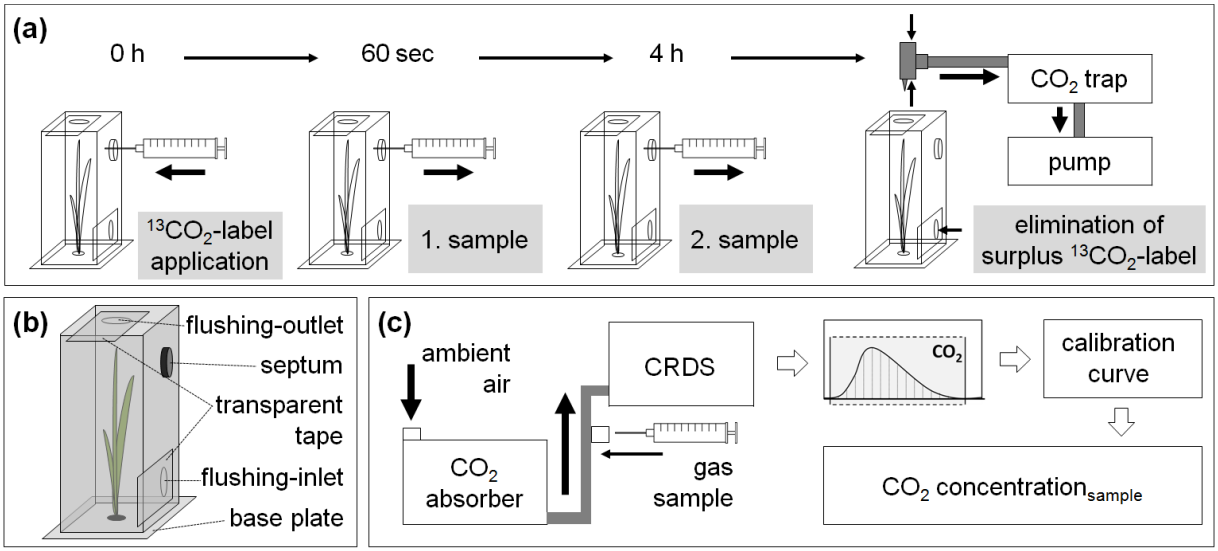

Fig. 5.3 Schematic of (a) $^{13}CO_2$ pulse-labeling procedure, (b) labeling-chamber and (c) cavity ring-down spectrometer, coupled with continuous flow injection (CRDS-CFI).

Australia), 1 ml of a 1:4 (ambient air : 99% $^{13}CO_2$, Linde AG, Munich, Germany) gas mixture was applied to each seedling (Fig. 5.3a). 60 s after label application a 2 ml gas sample was

extracted with another gas-tight syringe, which was instantaneously analyzed for the initial $^{13}CO_2$ concentration in the chamber, using cavity ring-down spectroscopy, coupled with a continuous flow injection (CRDS-CFI; see section 5.3.3). Before the sample was extracted, the air inside the chamber was mixed thoroughly by repeatedly pushing the piston of the syringe. After 4 h of label incubation, a second gas sample was extracted in order to determine the final $^{13}CO_2$ concentration. Before removing the labeling chamber, the remaining label was sucked from the chamber into a CO_2 trap, using a suction pipe ending in a sharp-edged T-piece nozzle (Fig. 5.3a), which perforated the tape sealing while plugging it into the flushing-outlet. Instantaneously, the tape sealing of the flushing-inlet was removed, thus flushing the chamber with fresh air. By subsequent closing of the outer end of the T-piece nozzle an air flow of ~15 l min^{-1} through the chamber was reached, which was maintained for 1 min, ensuring a complete elimination of surplus label. The reliability of this procedure was validated by 'indicator-plants' (*P. lanceolata*), that were kept next to the experimental plants throughout the whole experiment and were analyzed for ^{13}C during harvest (Brüggemann *et al.* 2011). In contrast to the labeled *P. lanceolata* seedlings, all 'indicator plants' exhibited natural ^{13}C abundance at the end of the experiment. Thus, (unintended) assimilation of label, that might potentially has been released by mistake, could be ruled out.

Fig. 5.4 *Plantago lanceolata* seedling, enclosed in a sealed, transparent polyethylene chamber for $^{13}CO_2$ pulse-labeling. See also Fig. 5.3.

5.3.3 Quantification of $^{13}CO_2$ assimilation using the 'CRDS-CFI' approach

A novel method, allowing the determination of CO_2 concentrations in small gas samples using a cavity ring-down spectrometer (CRDS, G2101-i Isotopic CO_2, PICARRO, INC., Santa Clara, CA) was applied. The CRDS analyzes gas with a continuous flow-rate of 25 ml min^{-1}, and is well suited for measurements of changes in CO_2 concentrations and isotopic signature of large samples (e.g., atmospheric or other open systems). When connecting a 'new' gas sample to the device, there is typically a temporary mixing with the preceding sample and it takes approx. 1 min until all previous gases are flushed away from the system, so that only the 'new' sample is measured, as reflected in a steady-state CO_2-'plateau'. However, small samples of only a few milliliters are insufficient to completely flush the system and thus produce 'peaks' rather than 'plateaus', thereby restricting the use of the device to large samples of at least 20 ml. However, since even small CO_2-'peaks' are well resolved, we compiled a calibration curve describing the relation between the CO_2 concentration of a sample and the integral of a CO_2-'peak'-area, resulting from a 2 ml injection of a corresponding subsample into the continuous flow to the CRDS. In order to reach the highest-possible resolution of the injection-induced CO_2-'peaks', an upstream CO_2 absorber was used to create a continuous CO_2-free gas flow (Fig. 5.3c). Calibration curves were compiled for both $^{12}CO_2$ and $^{13}CO_2$, revealing significant linear correlations ($r^2 > 0.999$; $p < 0.0001$) between 'peak'-integrals and the corresponding CO_2 concentrations. Using the equation of the obtained calibration curve, we were able to determine the CO_2 concentration of 2 ml gas samples. This new method will be referred to as 'cavity ring-down spectroscopy continuous flow injection' (CRDS-CFI) below.

By converting the determined concentrations to absolute molar amounts of $^{13}CO_2$ in the labeling chamber and subtracting initial from final values, we were able to calculate the exact amount of seedling-assimilated ^{13}C, which is rarely achieved in labeling exercises (Eq. 5.1):

$$^{13}C_{assimilated} = \left(c(^{13}CO_2)_{initial} \times \frac{V_{chamber}}{V_M} \right) - \left(c(^{13}CO_2)_{final} \times \frac{V_{chamber}}{V_M} \right) \qquad \text{Eq. 5.1}$$

[$^{13}C_{assimilated}$, the amount of seedling assimilated ^{13}C (µmol); $c(^{13}CO_2)_{initial}$ and $c(^{13}CO_2)_{final}$, the concentration of $^{13}CO_2$ in the labeling chamber (ppm) at the beginning and the end of the label incubation, respectively; $V_{chamber}$, the volume of the labeling chamber (0.1 l); V_M, the molar volume at T = 25 °C and p_{atm} = 1021.6 hPa (24.3 l mol^{-1}).]

In order to account for both label applications (seven and two days before harvest), $^{13}C_{assimilated}$-values of the two labeling events were added. Assimilation of natural $^{13}CO_2$ during labeling was negligible as label application led to ~1000-fold higher $^{13}CO_2$ concentrations as compared to natural abundance values.

5.3.4 Measurement of seedling-assimilated carbon in soil respiration and estimation of seedling-C transfer via mycorrhizal hyphae within the CMN

Subsequent to the second pulse-labeling (85 dap), 2 ml gas samples were extracted with a gas-tight syringe from the centre of each LP-compartment at a soil depth of 5 cm, in order to estimate the proportion of seedling-assimilated ^{13}C-label in soil respiration of each LP-compartment. CO_2 concentration of the extracted samples was determined using CRDS-CFI (as described in section 5.3.3). In addition, δ^{13}C of each sample was determined by averaging the CRDS-recorded δ^{13}C values over 15 s subsequent to the 'peak'-maximum, an episode in which the δ^{13}C reached steady state conditions. In contrast to CO_2 concentrations (see above), δ^{13}C values were not affected by dilution of the sample with the CO_2-free carrier gas. The proportion of ^{13}C-label in each gas sample was calculated, by relating *atom% ^{13}C* (Eq. 5.4; derived by substituting R_{sample} in Eq. 5.3 with Eq. 5.2, according to Heinemeyer *et al.* (2006)) of labeled samples to *atom% ^{13}C* of unlabeled samples (Eq. 5.5):

$$R_{sample} = \left(\left(\frac{\delta_{sample}}{1000} \right) + 1 \right) \times R_{ref} \qquad \text{Eq. 5.2}$$

$$atom\%^{13}C_{sample} = \frac{R_{sample} \times 100}{1 + R_{sample}} \qquad \text{Eq. 5.3}$$

$$atom\%^{13}C_{sample} = 100 \times \frac{\delta_{sample} + 1000}{90447.84 + \delta_{sample}} \qquad \text{Eq. 5.4}$$

$$atom\%^{13}C\,excess = atom\%^{13}C_{labeled} - atom\%^{13}C_{unlabeled} \qquad \text{Eq. 5.5}$$

[R_{sample} and R_{ref}, the ^{13}C:^{12}C ratios of sample and Vienna-Pee Dee Belemnite (V-PDB) reference (0.0111797; the reference used by CRDS for the ‰^{13}C calculation); δ_{sample}, the δ^{13}C of the sample; *atom% $^{13}C_{sample}$*, the percentage of ^{13}C in total C of the sample; *atom% ^{13}C excess*, the percentage of C in the sample, originating from the label application;

atom% $^{13}C_{labeled}$, *atom% $^{13}C_{sample}$* of labeled samples; *atom% $^{13}C_{unlabeled}$*, *atom% $^{13}C_{sample}$* of unlabeled samples.]

The *atom% ^{13}C excess* (i.e., the concentration of ^{13}C-label in the soil gas samples) was then related to the amount of label which was originally assimilated by the respective seedling (Eq. 5.6):

$$relative\ respiratory\ label\ partition = \left(\frac{atom\%\,^{13}C\ excess}{^{13}C_{assimilated}} \right) \times 1000 \qquad \text{Eq. 5.6}$$

[*relative respiratory label partition*, the percentage of assimilated label in 1 mmol of soil CO_2 (% mmol^{-1}); *atom% ^{13}C excess*, the percentage of C in the sample, originating from the label application; *$^{13}C_{assimilated}$*, the amount of ^{13}C-label, assimilated by the respective seedling during the (preceding) second label application (µmol).]

Calculations were exclusively based on label-assimilation during the second labeling pulse, since the proportion of label originating from the first labeling-pulse (5 days earlier), was negligible, as demonstrated in a preliminary test, where abundance of ^{13}C in soil respiration reached natural abundance values already after 24 h.

Even though we were not able to assess absolute label incorporation into soil respiration, the calculated relative respiratory label partition (RLP) in the soil gas enabled detection of (1) treatment-dependent differences in the total C-contribution of seedlings to soil respiration, (2) seedling-C transfer to LP-compartments via hyphae and (3) potential differences in the spatial distribution of seedling-assimilated C within the CMN.

The transport of seedling-C to LP-compartments via hyphal connections was estimated by comparing the RLP-values of 'connected' and 'disconnected' LP-compartments for each LP-species. Here, the RLP-values of disconnected LP-compartments reflected the proportion of RLP transferred to a LP-compartment via diffusion, which was subtracted from the RLP in corresponding intact LP-compartments to calculate carbon transfer via hyphae.

5.3.5 Final harvest and analysis of plant and fungal material

Two days after the second pulse-labeling (87 dap), the seedling-compartments were removed from the CMN-compartments (see below) and then all substrate was removed from the CMN-compartment and thoroughly mixed. A wet subsample was frozen and stored until analysis of soil N. Another subsample was dried at 40 °C for determination of soil P and hyphal extraction. All plants were divided into root and shoot biomass and oven-dried at 60 °C. Root

and shoot dry weights were recorded and root/shoot ratios were calculated. After separating a subsample of seedling roots for assessment of mycorrhizal colonization, shoot and root material of seedlings and 'indicator plants' was ground in a ball-mill (Retsch MM 301, Retsch, Haan, Germany) for analysis of elemental C and N, ^{13}C:^{12}C isotope ratios and P content.

A subsample of dried seedling roots was bleached in 10 % KOH at 90°C for 10 min, rinsed with deionized water and stained with an ink-acetic-acid solution (1:1:8 = ink : 10% acetic-acid : H_2O) at 90°C for 15 min, followed by a final, intense rinsing with deionized water (Phillips & Hayman 1970). The root fragments were then transferred to microscope slides and the percentage of root length colonized by AMF was estimated at x 250 magnification using a modified intersect method (McGonigle *et al.* 1990), scoring a minimum of 100 intersections per sample for the presence of hyphae, vesicles and arbuscules.

Seedling shoot P content was measured using high-temperature oxidation and colorimetrical quantification according to Watanabe & Olsen (1965). Dried plant material was ashed at 500°C for 4 h in a muffle furnace and subsequently 3 mg of ash was digested in 10% nitric acid. The extracts were diluted with bidestilled water and analyzed for orthophosphate concentration using flow injection analysis, as described in section 5.3.7. Tissue P concentration was calculated by relating the results to plant dry weight.

Extraradical (ER) hyphae were collected from the dried CMN-compartment substrate samples using a wet-sieving and decanting method, adapted to Bethlenfalvay & Ames (1987) and dried at 40°C.

ER hyphal and plant samples of seedlings and 'indicator plants' were analyzed for elemental C and N composition and $\delta^{13}C$ in the Laboratory of Isotope Biogeochemistry, Bayreuth Center of Ecology and Environmental Research (BayCEER). Samples were combusted in an elemental analyzer (NC 2500, CE Instruments, Milano, Italy) and analyzed in a continuous-flow isotope ratio mass spectrometer (delta plus, Thermo Fisher Scientific, Bremen, Germany). Recorded elemental N was used for calculation of seedling N content. Values of elemental C and $\delta^{13}C$ were used for quantification of ^{13}C-excess in seedlings and extraradical hyphae (see section 5.3.6).

5.3.6 Estimation of C allocation in seedling and fungal solid matter

Seedling C allocation to root, shoot and extraradical hyphae was quantified by calculating the respective label partitions on base of the IRMS-analyses of solid matter. First, the concentration of label in the respective sample was calculated by relating *atom% ^{13}C* of the labeled hyphal samples to *atom% ^{13}C* of the unlabeled roots of the 'indicator plants' (Eq. 5.7). After

relating the solid matter label concentration to the amount of seedling-assimilated label (Eq. 5.8), the label partition in each sample was calculated on base of the respective C concentrations and dry weights (Eq. 5.9), expressed as percentage of seedling-assimilated ^{13}C-label in the total sample.

$$label\ concentration = \frac{atom\%\,^{13}C_{labeled} - atom\%\,^{13}C_{unlabeled}}{100} \times 1000 \qquad \text{Eq. 5.7}$$

$$relative\ label\ \ partition = \frac{label\ concentration}{^{13}C_{assimilated}} \times 100 \qquad \text{Eq. 5.8}$$

$$absolute\ label\ \ partition = relative\ label\ \ partition \times c(C) \times W \qquad \text{Eq. 5.9}$$

[*label concentration*, the percentage of ^{13}C-label in sample-C (mmol mol^{-1}); *atom%* $^{13}C_{labeled}$, the percentage of ^{13}C in sample-C of labeled material; *atom%* $^{13}C_{unlabeled}$, the percentage of ^{13}C in sample-C of unlabeled material ('indicator plants'); *relative label partition*, the percentage of seedling-assimilated label in sample-C (% mmol^{-1}); $^{13}C_{assimilated}$, the amount of seedling-assimilated ^{13}C-label (µmol); *absolute label partition*, the (% sample^{-1}); *c(C)*, C-concentration in the sample (mmol g^{-1}); *W*; sample dry weight (g).]

Label partitions were calculated for seedling roots and shoots. By subtraction of the root and shoot partition from the amount of seedling-assimilated label, the seedling C-losses ('lost' label partition; i.e., the difference between assimilated label and label detected in plant tissue) were calculated. For calculation of the CMN label partition (percentage of seedling-assimilated label in extraradical hyphae), hyphal dry weight was calculated on base of hyphal biovolume (mm³ per CMN-compartment) after Bakken & Olsen (1983), according to Jakobsen & Rosendahl (1990).

5.3.7 Quantification of soil N and P

Plant available nitrate-N and phosphate-P concentrations were determined in the CMN-substrate samples from the beginning (60 dap) and from the end (87 dap). P was extracted using a modified calcium-acetate-lactate (CAL) extraction method according to Schüller (1969). A suspension of 5 g of dried substrate and 50 ml CAL solution (77 g calcium lactate, 39.5 g calcium acetate, 89.5 ml 100% acetic acid l^{-1}) was shaken for 90 min, then centrifuged at 3000 rpm for 3 min. N was extracted by suspending 15 g of fresh (frozen) substrate in

30 ml 0.1 M $CaCl_2$. Samples were shaken for 60 min, then centrifuged at 3000 rpm for 3 min. The supernatants of both P- and N-extracts were passed through a glass fiber filter (1 µm pore size) using a Luer syringe. Concentration of N and P in the extracts was measured colorimetrically at 546 and 880 nm, respectively, using flow injection analysis (FIA-Lab II, MLE GmbH, Dresden, Germany).

5.3.8 Statistical analyses

Statistical analyses were performed using Statistica 6.0 (StatSoft Inc., Tulsa, USA). Data were tested for normal distribution (Shapiro-Wilk test) and homogeneity of variances (Brown-Forsythe test). Data that did not satisfy the assumptions of normal distribution were square root or log transformed prior to analysis. Repeated-measures one-way ANOVA was performed on data of initial and final extraradical hyphal density, soil N and soil P concentration, testing for effects of time, treatment and for cross-interaction between the two factors. One-way ANOVA was performed on data of seedling total dry weight, shoot N:P ratio, shoot N and P content, shoot N and P concentration, label partitions in solid matter of seedling and mycelium and on the average label partitions in soil respiration, testing for effects of treatments. Two-way ANOVA was performed on large plant dry weight, root fraction and ^{13}C-label partition in soil respiration, testing for effects of both treatment and large plant species and for a cross-interaction between the two factors. When ANOVA revealed significant main effects, Fisher's LSD post-hoc test was applied for pairwise comparisons. Student's *t*-test was performed for pairwise comparisons between initial and final soil P concentrations. Kruskal-Wallis one-way ANOVA on ranks was performed on colonization level, arbuscule and vesicle abundance. When main effects were significant, Mann-Whitney U tests were performed for pairwise comparisons. Spearman-*R* was determined for correlations of seedling label partitions with mycelium size and hyphal label partition, and for correlations of respiration label partition with 'lost' partition, mycelium size, seedling colonization and seedling root dry weight.

5.4 Results

5.4.1 Fungal growth

Mycorrhizal seedling root colonization was significantly lower in the 'no CMN' treatment than in all other treatments ($p < 0.001$) (Tab. 5.1). There were no significant differences between all other treatments.. Colonization level in the 'CMN -all' treatment was slightly lower than in those treatments, where large plant CMN-connections were retained, although this difference was not significant ($p = 0.09 - 0.29$). In line with the colonization levels, there was a visible trend of lower abundances of vesicles and arbuscules in 'no CMN' as compared to the other treatments. However, treatments had no significant effect on the abundance of these structures.

Tab. 5.1 Percentage of seedling root length colonized by arbuscular mycorrhizal fungi and abundance of arbuscules and vesicles. Different letters indicate significant differences between treatments within each of the three parameters at $p = 0.05$ (Kruskal-Wallis-ANOVA on ranks; Mann-Whitney U-test; n.s. = no significant main effects). Means ± SE, n = 6.

Treatment	Colonization (%)	Vesicles (%)	Arbuscules (%)
no CMN	53 ± 11 b	29 ± 8 n.s.	22 ± 5 n.s.
full CMN	92 ± 3 a	47 ± 4 n.s.	34 ± 3 n.s.
CMN -*Hp*	93 ± 3 a	41 ± 6 n.s.	37 ± 3 n.s.
CMN -*Pl*	92 ± 4 a	46 ± 7 n.s.	35 ± 4 n.s.
CMN -*Cc*	96 ± 1 a	50 ± 4 n.s.	40 ± 3 n.s.
CMN -*Fp*	94 ± 2 a	43 ± 5 n.s.	37 ± 6 n.s.
CMN -all	85 ± 3 a	46 ± 4 n.s.	34 ± 5 n.s.

Initial extraradical hyphal densities in the CMN-compartment (60 dap) were significantly lower ($p < 0.05$) in the 'no CMN' treatment than in all other treatments (Fig. 5.5). A significant increase ($p < 0.05$) of hyphal densities during the establishment period was found in the treatments 'full CMN', 'CMN -*Fp*', 'CMN -*Cc*' and 'CMN -*Hp*'. In these treatments, final hyphal densities (87 dap) significantly ($p < 0.05$) exceeded the values of the 'CMN -all' treatment. In contrast, final hyphal density in the 'CMN -*Pl*' treatment was neither significantly different from the initial 'CMN -*Pl*' nor from the final 'CMN -all' value. Moreover, final hyphal density in the 'CMN -*Pl*' treatment was (nearly significantly; $p = 0.062$) lower than in the 'full CMN' treatment, whereas differences to the 'full CMN' treatment were less pronounced in 'CMN -*Fp*', 'CMN -*Cc*' and 'CMN -*Hp*'. Hyphal densities in the 'no CMN' treatment increased significantly during the establishment period, whereas a significant decrease in hyphal density was found in the 'CMN -all' treatment ($p < 0.05$). Final hyphal densities were not significantly different between 'CMN -all' and 'no CMN'.

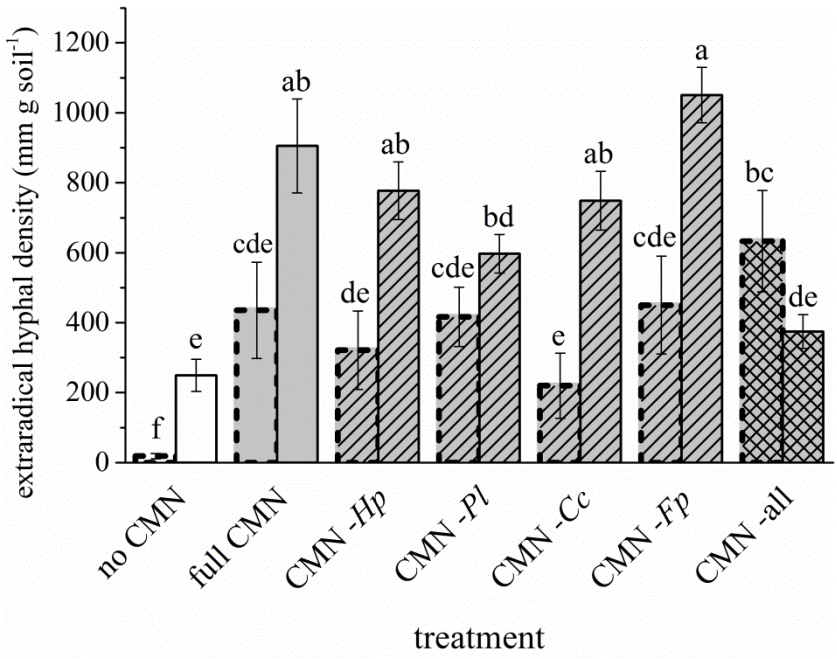

Fig. 5.5 Initial (beginning of the establishment period (60 dap); dotted frames) and final (end of the experiment (87 dap); solid frames) hyphal densities in the CMN-compartments of the different treatments ('no CMN' - white; 'full CMN' - grey; 'CMN -*Hp*, -*Pl*, -*Cc*, -*Fp*' - hatched; 'CMN -all' - checked). Different letters indicate significant differences between initial and final values and between treatments at $p = 0.05$ (repeated measures ANOVA; Fisher's LSD post-hoc test). Means ± SE, $n = 6$.

5.4.2 Plant growth

Total seedling dry weight did not differ between the treatments, except for the 'CMN -all' treatment, where seedling dry weight was significantly higher than in all other treatments ($p < 0.05$; Fig. 5.6). Large plant dry weight and root fraction was significantly different between the four species (Tab. 5.2, 5.3). *H. pilosella* and *F. psammophila* exhibited the lowest and the highest total dry weight, respectively, while *P. lanceolata* and *C. canescens* showed intermediate values (Tab. 5.2). *H. pilosella* showed the lowest root proportion, whereas *P. lanceolata* and *F. psammophila* had the highest values. Root fraction of *C. canescens* was intermediate (Tab. 5.2). Treatments had no significant effect on large plant dry weights and root fractions (Tab. 5.3).

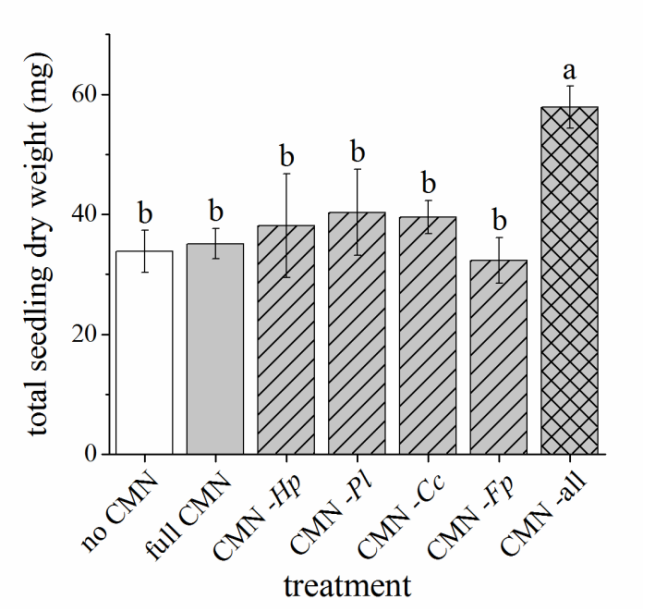

Fig. 5.6 Average total dry weight of seedlings, dependent on treatment ('no CMN' - white; 'full CMN' - grey; 'CMN -*Hp*, -*Pl*, -*Cc*, -*Fp*' - hatched; 'CMN -all' - checked). Different letters indicate significant differences between treatments at $p = 0.05$ (ANOVA; Fisher's LSD post-hoc test; n.s. = no significant main effects). Means ± SE, $n = 6$.

Tab. 5.2 Average total dry weight and root fraction of large plants. Different letters indicate significant differences between large plant species at p = 0.05 (ANOVA; Fisher's LSD post-hoc test). Means ± SE, n = 42.

Large plant species	Total dry weight	Root fraction
Hieracium pilosella	0.177 ± 0.006 d	0.427 ± 0.009 c
Plantago lanceolata	0.290 ± 0.007 b	0.591 ± 0.006 a
Corynephorus canescens	0.256 ± 0.006 c	0.504 ± 0.008 b
Festuca psammophila	0.383 ± 0.013 a	0.581 ± 0.010 a

Tab. 5.3 Results of two-way ANOVAs on large plant (LP) dry weight, large plant root fraction and ^{13}C-label partition in soil respiration of large plant compartments. Sums of squares (*SS*), *F*- and p-values are given for the factors 'treatment' and 'large plant species' and for cross-interaction between the two factors. Boldface values indicate significant effects.

Large plant parameter	Factor	df	*SS*	*F*	p
dry weight	treatment	6	0.017	0.862	0.525
	LP species	3	0.864	89.909	< **0.0001**
	treatment X LP species	18	0.024	0.414	0.983
root fraction	treatment	6	0.015	0.86	0.527
	LP species	3	0.685	79.92	< **0.0001**
	treatment X LP species	18	0.079	1.54	0.087
^{13}C-label partition in soil respiration	treatment	6	75.805	42.245	< **0.0001**
	LP species	3	1.662	1.852	0.141
	treatment X LP species	18	14.522	2.698	< **0.001**

5.4.3 Seedling C allocation

The relative hyphal label partition (percentage of seedling-assimilated label per hyphal C) was not different between the treatments (F = 0.698; SS = 0.012; p = 0.653, Fig. 5.7a). The (absolute) hyphal label partition (percentage of seedling-assimilated label per total mycelium) was highest in the 'CMN -*Fp*' treatment and lowest in the 'no CMN' and 'CMN -all' treatment (Fig. 5.7b). Hyphal label partitions in the treatments 'full CMN', 'CMN -*Hp*', 'CMN -*Pl*' and 'CMN -*Cc*' showed intermediate values.

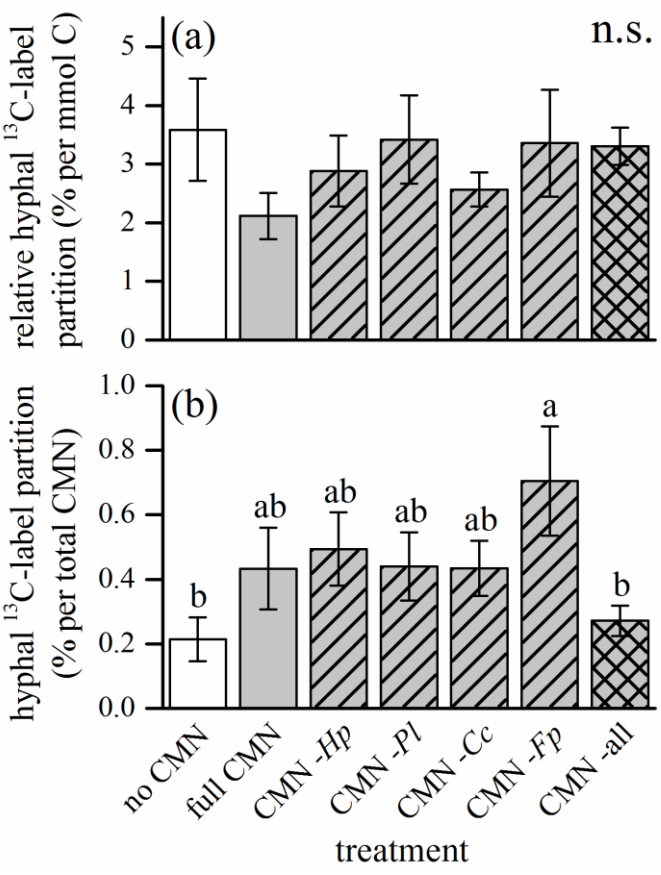

Fig. 5.7 Percentage of seedling-assimilated ^{13}C-label in extraradical mycorrhizal hyphae in the CMN-compartment, as calculated per (a) mmol hyphal C and (b) total mycelium, dependent on treatment ('no CMN' - white; 'full CMN' - grey; 'CMN -*Hp*, -*Pl*, -*Cc*, -*Fp*' - hatched; 'CMN -all' - checked). Different letters indicate significant differences between treatments at p = 0.05 (ANOVA; Fisher's LSD post-hoc test; n.s. = no significant main effects). Means ± SE, n = 6.

The relative respiratory label-partition in LP-compartments (RLP; expressed as percentage of seedling-assimilated label per mmol soil CO_2) was significantly different between the treatments (Tab. 5.3, Fig. 5.8). The lowest average RLP-value per pot was found in the 'no CMN' treatment (Fig. 5.8), whereas 'CMN -all' treatments exhibited significantly higher proportions. In comparison, the proportion of seedling C in soil respiration was significantly higher in pots with retained large plant connections ('full CMN' and 'specific exclusion'; Fig. 5.8). The label partition, measured in each LP-compartment, was not dependent on the respective large plant species (Tab. 5.3). This was obvious by lacking differences between RLP-values of LP-compartments in the treatments 'no CMN', 'full CMN' and 'CMN -all'. Significant differences between LP-compartments in 'full CMN' and 'specific exclusion' pots were due to reduced label partitions in the 'disconnected' LP-compartments, whereas the (three) 'connected' LP-compartments were in no case different from each other (Fig. 5.8).

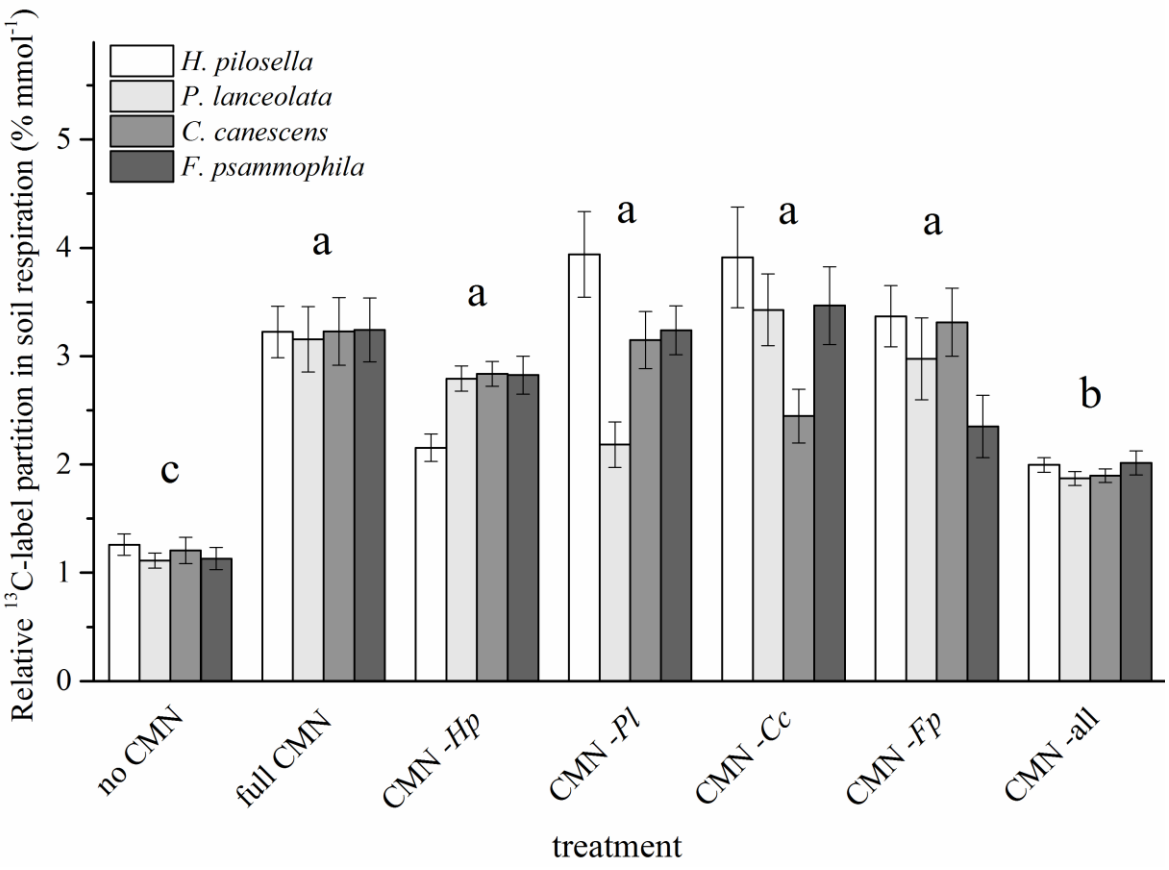

Fig. 5.8 Relative ^{13}C-label partition in large plant compartment soil respiration (RLP), expressed as percentage of ^{13}C-label assimilated by the seedling during the second labeling pulse per mmol soil CO_2, dependent on treatment (x-axis) and large plant compartments (legend). Different letters indicate significant differences between the average 'per pot' values within each treatment at p = 0.05 (ANOVA; Fisher's LSD post-hoc test). Means ± SE, n = 6. See Tab. 5.3 for ANOVA results.

The average relative label partition in soil respiration (per pot) was significantly positively correlated with the respective 'lost' label partition, mycelium size and seedling root colonization, whereas it was not correlated with seedling root dry weight (Tab. 5.4).

Tab. 5.4 Results of tests on correlation between ^{13}C-label partition in soil respiration per pot (average value of the four large plant compartments) and the corresponding 'lost' label partition, mycelium size, seedling colonization level and root dry weight. Spearman-*R* and p-values are given. Significant correlations at p = 0.05 are indicated by boldface *P*-values. n = 42.

Correlation	*Spearman- R*	p
resp. label partition x 'lost' label partition	0.530	< **0.001**
resp. label partition x mycelium size	0.615	< **0.0001**
resp. label partition x seedling colonization	0.505	**0.0017**
resp. label partition x seedling root dry weight	-0.050	0.768

Tab. 5.5 Results of tests on correlation of seedling shoot ^{13}C-label partition, root ^{13}C-label partition and 'lost' ^{13}C-label partition (difference between the amounts of assimilated and detected ^{13}C-label in seedling tissue) with mycelium size. Spearman-*R* and p-values are given. Significant correlations at p = 0.05 are indicated by boldface p-values. n = 42.

Correlation	*Spearman- R*	p
lost label partition x mycelium size	0.636	< **0.0001**
root label partition x mycelium size	-0.468	**0.0040**
shoot label partition x mycelium size	-0.494	**0.0022**

A significant transfer of C to LP-compartments via hyphal connections was found in all CMN-connected LP-compartments, as revealed by consistently significant differences ($p < 0.01$) to the RLP-values of 'disconnected' LP-compartments (Fig. 5.9). Although C-transfer to *H. pilosella* LP-compartments seemed to be slightly higher than to other LP-compartments, this difference was not significant. However, C-transfer to LP-compartments via hyphae was in no LP-species affected by exclusion of other LP-species, as indicated by lacking significant differences between hyphal C-transfer in 'full CMN' and the different 'specific exclusion' treatments (Fig. 5.9). Moreover, the total hyphal C-transfer to LP-compartments within each pot was not significantly different between the treatments, although it was slightly lower in 'CMN -*Hp*' than in the other treatments.

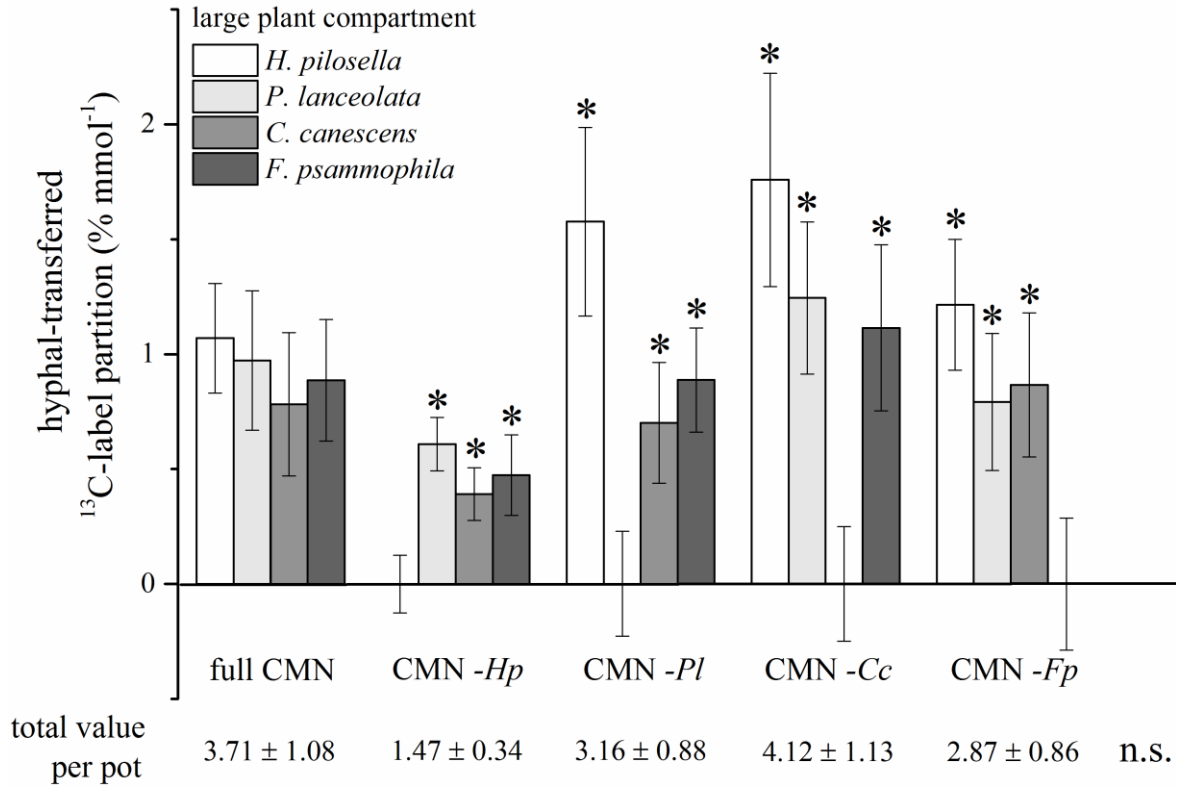

Fig. 5.9 Relative transfer of seedling-C to the four large plant compartments (LP; see legend) via hyphal connections, calculated by subtracting the relative ^{13}C-label partition in soil respiration (RLP) of 'disconnected' LP-compartments (pure diffusion, no hyphal C-transfer) from RLP-values of 'connected' LP-compartments of the respective LP-species (see also Fig. 5.8), dependent on treatment (x-axis) and large plant compartment (legend). Means ± SE, n = 6. Values below X-axis represent the mean sum of hyphal C-transfer per pot ± SE, n = 6. Asterisks above bars represent significant differences between RLP-values of 'connected' LP-compartments to the respective 'disconnected' LP-compartment.

The seedling C-losses ('lost' label partition; the difference between the amounts of assimilated and detected ^{13}C-label in seedling tissue; Fig. 5.10c) was lowest in the 'no CMN' seedlings (~30%), intermediate in the 'CMN-all' treatment and highest those treatments, where CMN-connections of three or all four large plants were retained (~50%). Within these treatments, seedling C-losses were marginally higher in 'CMN -*Fp*' than in the other treatments (p = 0.30 - 0.42). The root label partition was highest in 'no CMN' seedlings (Fig. 5.10b).

'Lost' label partitions were positively correlated with mycelium size, whereas root and shoot label partitions were negatively correlated with mycelium size (Tab. 5.5).

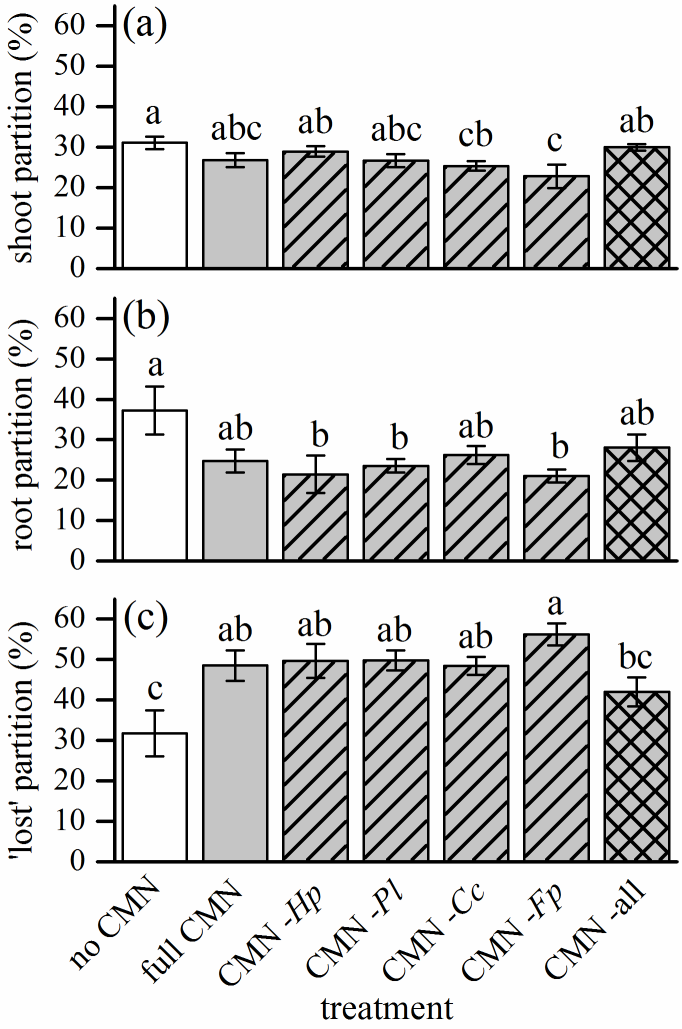

Fig. 5.10 Seedling (a) shoot ^{13}C-label partition, (b) root ^{13}C-label partition and (c) 'lost' ^{13}C-label partition (difference between the amounts of assimilated and detected ^{13}C-label in seedling tissue), expressed as percentage of assimilated ^{13}C-label, dependent on treatment ('no CMN' - white; 'full CMN' - grey; 'CMN -*Hp*, -*Pl*, -*Cc*, -*Fp*' - hatched; 'CMN -all' - checked). Different letters indicate significant differences between treatments at p = 0.05 (ANOVA; Fisher's LSD post-hoc test). Means ± SE, n = 6.

5.4.4 N and P in soil and seedling tissue

Shoot N:P ratio was significantly higher in the 'no CMN' treatment than in all other treatments (~25; $p < 0.002$), whereas only marginal differences ($p > 0.05$) were found between these

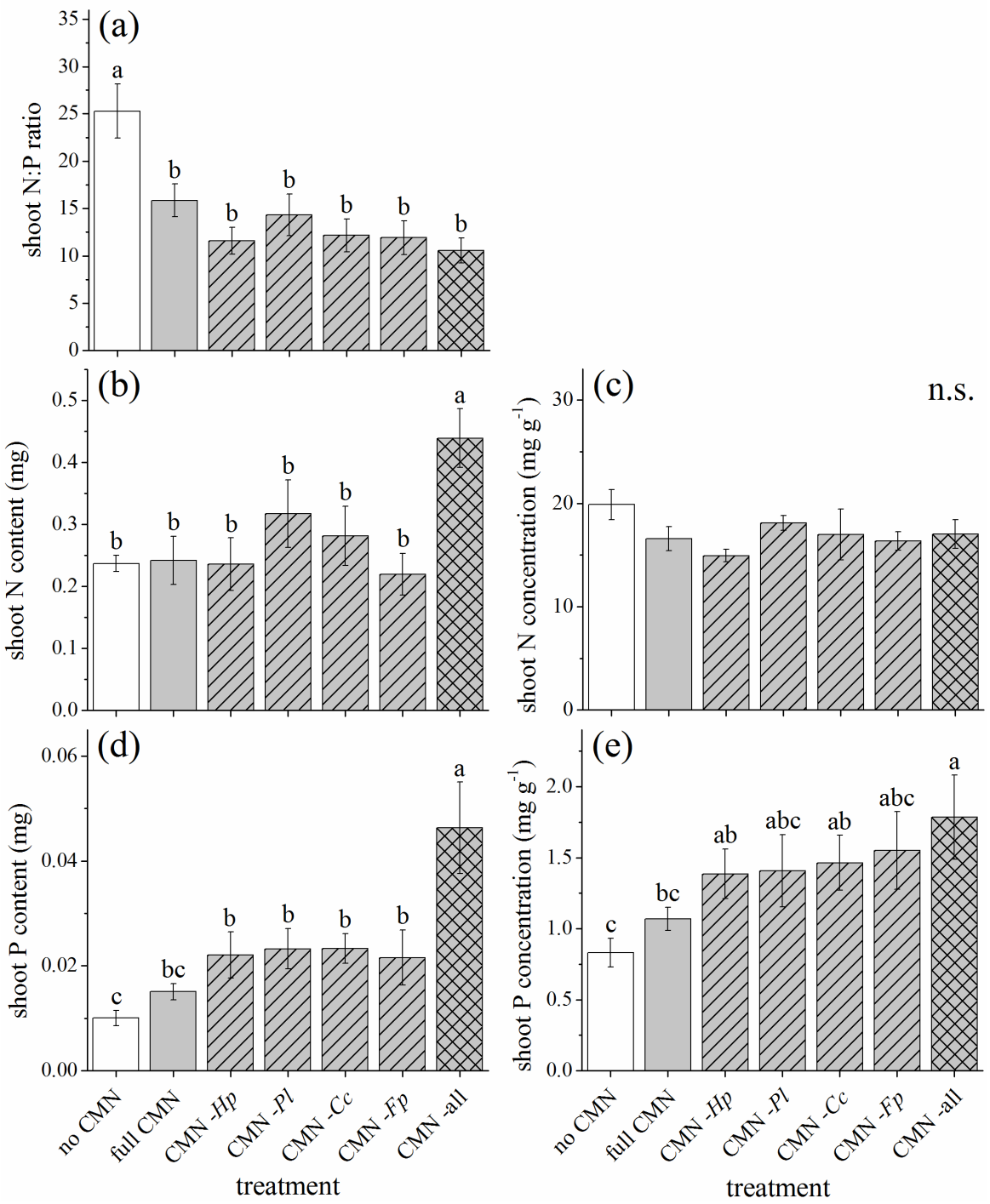

Fig. 5.11 Seedling (a) shoot N:P ratio, (b) shoot N content, (c) shoot N concentration, (d) shoot P content and (e) shoot P concentration of the different treatments ('no CMN' - white; 'full CMN' - grey; 'CMN -*Hp*, -*Pl*, -*Cc*, -*Fp*' - hatched; 'CMN -all' - checked). Different letters indicate significant differences between treatments at $p = 0.05$ (ANOVA; Fisher's LSD post-hoc test; n.s. = no significant main effects). Means ± SE, n = 6.

treatments, with values of ~10 in 'CMN -*Hp*', '*CMN -Cc*', 'CMN -*Fp*' and 'CMN -all', and values of ~15 in 'full CMN' and 'CMN -*Pl*' (Fig. 5.11b). Treatment-dependent differences in shoot N:P ratio were mainly driven by different P concentrations, that were noticeably lower in 'no CMN' and 'full CMN' treatments, whereas disruption of large plant CMN connections resulted in enhanced shoot P concentrations, with the highest value in the 'CMN -all' treatment (Fig. 5.11f). In contrast, N concentrations did not differ between the treatments (Fig. 5.11d). The absolute shoot N content was driven by seedling dry weight (Fig. 5.6, 5.11b), whereas P content correlation with seedling biomass was less pronounced (Fig. 5.6, 5.11e).

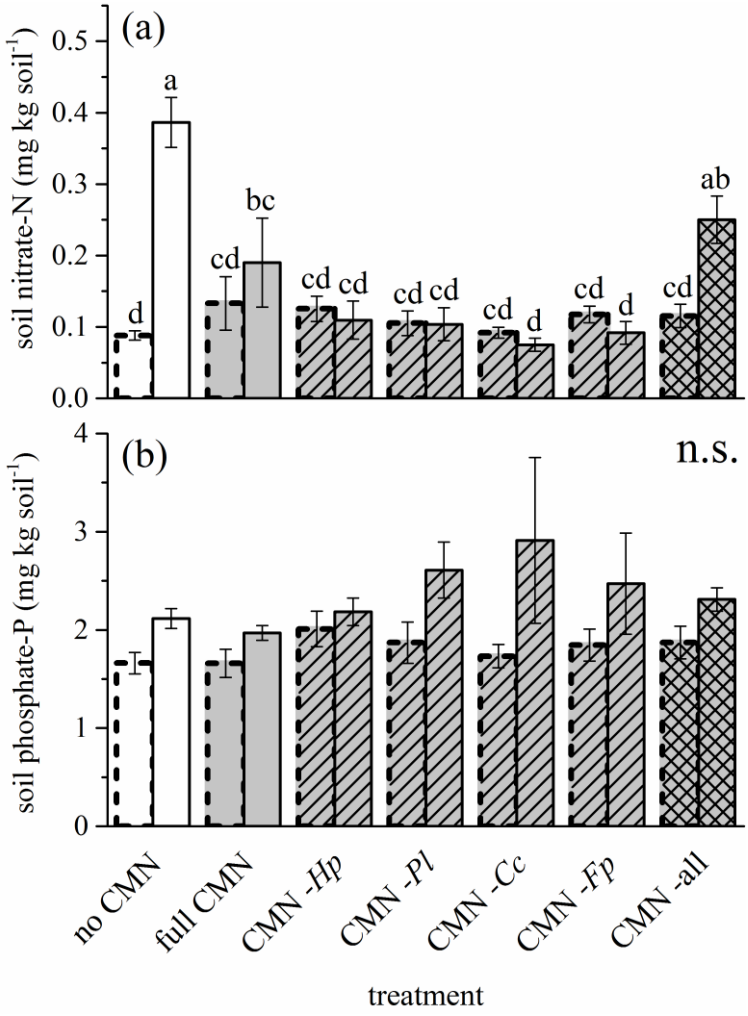

Fig. 5.12: Initial (beginning of the establishment period (60 dap); dotted frames) and final (end of the establishment period (87 dap); solid frames) soil (a) nitrate-N and (b) phosphate-P concentrations of the different treatments ('no CMN' - white; 'full CMN' - grey; 'CMN -*Hp*, -*Pl*, -*Cc*, -*Fp*' - hatched; 'CMN -all' - checked). Different letters indicate significant differences between initial and final values and between treatments at p = 0.05 (repeated measures ANOVA; Fisher's LSD post-hoc test; n.s. = no significant main effects). Means ± SE, n = 6.

In 'no CMN' and 'CMN -all', soil N concentration was significantly increased during the establishment period, as indicated by higher final than initial values, whereas initial and final values were not significantly different in the other treatments (Fig. 5.12a). In contrast, soil P concentration was on average increased during the establishment period (Fig. 5.12b), as indicated by a significant effect of the factor 'time' ($p < 0.0001$). However, P concentration was not dependent on treatment ($p = 0.5503$), and the extent of P increase during the establishment period was not different between treatments ($p = 0.7084$).

5.5 Discussion

5.5.1 Existence of a CMN and net seedling facilitation

Our first basic assumption that large plants and seedlings were connected by a CMN, was validated by data on seedling root colonization (Tab. 5.1). Mycorrhizal hyphae, spreading from large plant roots, efficiently increased colonization levels of seedlings in all treatments above 'no CMN' values. In contrast, the on average lower and more variable colonization level in 'no CMN'-seedlings indicated a slower colonization, since previous experiments showed infection of *P. lanceolata* plants by *R. intraradices* to consistently result in final colonization levels of almost 100% (Ch. 2-4). This delay in colonization can be explained by mycorrhizal infection being completely dependent on C supply by the seedling. Indeed, mycorrhizal root colonization based on dormant fungal infection units has been shown to be a comparatively inefficient and slow way of infection (e.g., McGee 1989; Braunberger *et al.* 1994; Merryweather & Fitter 1998), thus being unfavorable for rapid seedling establishment. The results are in line with other studies, demonstrating that seedling root colonization may be promoted by CMNs, provided by neighboring plants (Read *et al.* 1985; Eissenstat & Newman 1990; Read 1992; Olsson *et al.* 2002; Leake *et al.* 2004; Simard & Durall 2004). Further support for the existence of a functional CMN between large plants and seedlings is given by the fact that seedling colonization was slightly higher when large plant CMN-connections were retained ('specific exclusion' and 'full CMN') than when all connections were disrupted ('CMN -all'), with seedling C supply potentially not being sufficient to maintain the CMN established by large plants.

Our second basic assumption of net seedling facilitation via CMN was not confirmed, as seedling biomass in the 'full CMN' and the four 'specific exclusion' treatments was not higher than in the 'no CMN' treatment (Fig. 5.6), thus indicating a neutral net CMN-effect on seedling growth. However, the noticeably increased seedling biomass in the 'CMN -all'

treatment indicates that lacking net facilitation in the 'full CMN' and the 'specific exclusion' treatments cannot be simply explained by lacking CMN-effects. In contrast, this result points towards a positive 'inoculation effect', resulting from CMN-provision by large plants, which, however, was overlaid by negative CMN-effects due to retaining large plant CMN-connections. The potential reasons for positive and negative CMN-effects are discussed in the following sections. Moreover, contrasting our expectation, seedling growth did not differ between the 'specific exclusion' treatments. Possible reasons for these results are discussed below.

5.5.2 Maintenance of the CMN by highly mycotrophic 'key species'?

As intended, severing of hyphae prevented large plants from establishing an extraradical mycelium inside the CMN-compartment, as shown by hyphal densities close to zero in the 'no CMN' treatment at the beginning of the establishment period (Fig. 5.5). In all other treatments, undisturbed mycelial growth during large plant precultivation resulted in clearly higher initial hyphal densities, indicating transfer of large plant C to the CMN-compartment during the precultivation period. In addition, the dependence of the established CMN on large plant C supply ('specific exclusion' and 'full CMN') was shown by ongoing CMN growth during the facilitation period (except for 'CMN -*Pl*', see below), which was in marked contrast to the 'CMN -all' treatment, where hyphal densities declined during that time. These results confirm earlier observations that mycelium growth and maintenance is highly dependent on the extent of recent C supply by the hosts connected to the CMN (Jakobsen & Rosendahl 1990; Staddon *et al.* 1998; Heinemeyer *et al.* 2006), with the small seedlings presumably not being able to maintain large CMNs.

In a previous experiment, a CMN established and maintained by the highly mycotrophic species *H. pilosella* was shown to have a high facilitation potential (see Ch. 4). Therefore, we hypothesized that *H. pilosella* might invest disproportionately high amounts of C to a CMN, thus being a 'key species' for CMN-maintenance. However, since in the present experiment CMN-growth was not reduced due to disconnection of *H. pilosella* large plants, compared to the 'full CMN' treatment (Fig. 5.5), this hypothesis has to be rejected. In contrast, excluding *P. lanceolata* large plants from the CMN inhibited ongoing CMN-growth during the establishment period, resulting in decreased final hyphal densities. This result indicates that CMN-growth was predominantly based on C contributions by the highly mycotrophic *P. lanceolata*, and supports the hypothesis of van der Heijden & Horton (2009) that in a plant community, there might be certain 'key species', that are of particular importance for CMN-

maintenance. The comparatively low contributions by *H. pilosella* to CMN-maintenance might be explained by the relatively low biomass of *H. pilosella* large plants as compared to the other LP species (Tab. 5.2), indicating a lower total C budget, which probably is a key factor for the extent of absolute C supply to a CMN (van der Heijden & Horton 2009; Merrild *et al.* 2013). Interestingly, exclusion of *F. psammophila*, which exhibited higher biomass than all other large plant species (Tab. 5.2), thus potentially possessing the highest total C budget, did not lead to decreased CMN size. In contrast, this treatment revealed the highest final hyphal density of all 'specific exclusion' treatments, indicating that a large plant 'community' of *H. pilosella*, *P. lanceolata* and *C. canescens* is the most 'powerful' combination for CMN growth. These results are in line with results of an earlier study, where *F. psammophila* had been revealed to produce almost no mycelium compared to the other three species (Ch. 3). Based on these results, we conclude that both, plant size and the species-specific mycotrophy level are important determinants for the relative importance of a host plant for CMN-maintenance. We conclude that productive plants with high mycotrophy level may often be 'key species' for CMN-maintenance.

5.5.3 Seedling CMN-costs

Our basic assumption that *P. lanceolata* seedlings allocate C to a CMN maintained by large plants was confirmed by significant amounts of ^{13}C-label found in extraradical hyphae (Fig. 5.7a), indicating that, irrespective of large plant C-supply, there were CMN-costs to seedlings. However, in contrast to our expectation, seedling CMN-costs were not reduced but increased by retaining large plant CMN-connections. First, the absolute amount of seedling-C, allocated to the extraradical mycelium, was consistently increased when large plant CMN-connections were retained, even though only significantly higher in mycelia of 'CMN -Fp' (Fig. 5.7b). However, since these higher absolute amounts of seedling C in the CMN were solely caused by higher hyphal densities (Fig. 5.5), whereas the relative seedling-C allocation to the CMN (i.e., the proportion of C allocated per fungal biomass) was more or less constant (Fig. 5.7a), we suggest that seedling CMN-costs are predominantly driven by mycelium size.

However, estimations on CMN-costs to the seedlings based on seedling C incorporated in hyphae inside the CMN-compartment may not reflect the total CMN-costs to the seedling, as in case of retained CMN-connections, the CMN was also extended to LP-compartments. It has to be considered that retaining large plant CMN-connections provided 75% ('specific exclusion' treatments) and 100% ('full CMN' treatment) extra space for CMN extension, pointing towards additional CMN-incorporated seedling C. Moreover, presence of

roots in these compartments should be accompanied by occurrence of intraradical AMF-structures, potentially representing additional sinks for seedling C. This is supported by the generally increased proportion of label in soil respiration (RLP) of 'full CMN' and 'specific exclusion' treatments, thus confirming increased seedling C-investment in these treatments (Fig. 5.8, Tab. 5.4). This is in contrast to our basic assumption that C-supply by large plants would lead to reduced seedling C-costs. Although it is widely accepted that seedling C-costs for root colonization may be reduced by interlinking to a CMN (e.g., Brundrett *et al.* 1985; Grime *et al.* 1987; Rosewarne *et al.* 1997), our results point towards increased seedling C-costs for CMN-maintenance, which up to now had not yet been investigated. This observation, however, is another argument for CMN-size being the driving factor for the extent of seedling CMN-costs. Relating the species-specific RLP-values of CMN-connected LP-compartments to those of the corresponding disconnected LP-compartments gave powerful evidence for transfer of seedling-C via hyphal connections to LP-compartments to be the main reason for increased seedling C drain by the CMN (Fig. 5.9). However, there were no significant differences in the amounts of seedling C transferred to large plant compartments, suggesting a minor relevance of the presumably different C source strength of large plants.

Besides the unexpected fact, that CMN-costs to the seedling were not reduced but increased due to retaining large plant CMN-connections, there were no differences in seedling CMN-costs within the different 'specific exclusion' treatments. Thus, our second hypothesis that exclusion of *H. pilosella* would increase seedling CMN-costs, has to be rejected. Even though *P. lanceolata* has been identified as a 'key species' for CMN-growth, suggesting high C-supply to the CMN (as discussed in section 5.5.2), seedling CMN-costs were not altered due to exclusion of this large plant species. Nevertheless, we can conclude that the extension of a CMN by large plants may lead to increased seedling CMN-costs. This finding is further supported by higher seedling 'C losses', when large plant CMN-connections were retained (Fig. 5.10c). Indeed, 'C losses' were strongly correlated with mycelium size (in the CMN-compartment, Tab. 5.5) and the amount of seedling-C in soil respiration (Tab. 5.4). On base of these results, it cannot be ruled out that increased CMN-costs represent a negative CMN-effect (besides competition for nutrients, as discussed in section 5.5.4), overlaying the positive 'inoculation effect' and may partially be responsible for lacking net facilitation.

Although 'C-losses' include any C-allocation to extraradical fungal structures, this measure does not account for C allocation to intraradical fungal structures. Partitioning seedling root and shoot C-allocation revealed a comparatively high proportion of ^{13}C-label in the roots of 'no CMN'-seedlings (Fig. 5.10b), indicating an increased C-investment into

intraradical AMF-structures, probably resulting from the lacking colonization support by large plants. This is supported by the fact that lower 'C losses' in 'no CMN'-seedlings were not translated to a positive growth response. In contrast, the lower C-allocation to roots in case of disrupted large plant CMN-connections might indicate lower C-costs to the seedling for root colonization, suggesting that a positive 'inoculation effect' is not only based on enhanced nutrient uptake but might partially be due to 'saved' C (e.g., Brundrett *et al.* 1985; Grime *et al.* 1987; Rosewarne *et al.* 1997). This suggests that total mycorrhizal C-costs to seedlings, including costs of both intra- and extraradical fungal structures, are not strongly affected by the increased C-allocation to extraradical hyphae. In contrast, a trade-off between C-allocation to intra- and extraradical structures is likely, with the net effect on seedling C-investment being determined by decreased C-costs for intraradical structures with simultaneously larger C-costs for extraradical structures and vice versa, depending on the presence of large plants. Although we were not able to compare C-saving due to CMN-supported inoculation and C-losses due to CMN-costs quantitatively, C-costs and C-benefits might be balanced, suggesting that total mycorrhizal C-costs to the seedling might be of minor importance for CMN-mediated seedling facilitation by neighboring large plants.

5.5.4 CMN-mediated competition for nutrients

The positive 'inoculation effect' on seedling growth due to provision of a CMN by large plants was obviously caused by enhanced P-nutrition. According to Koerselman & Meuleman (1996), who proposed that N:P ratios < 14 generally indicate N limitation and N:P ratios > 16 indicate P limitation, the noticeably high N:P ratios of ~25 in 'no CMN'-seedlings (Fig. 5.11b) indicate a strict P-limitation. However, this cannot be explained by insufficient availability of soil P, considering that P demand of plants is generally about 10-fold lower than N demand (Marschner 2003) and soil P was available in much larger amounts than soil N (Fig. 5.12). Thus, P-limitation was most likely due to a lacking promotion of root colonization by a CMN in 'no CMN'-seedlings, which is in agreement with the results presented in Ch. 3. Thus, we conclude that *P. lanceolata* seedlings, germinating on bare sand, might principally benefit from presence of a CMN, as this mechanism obviously accelerates overcoming P-limitation, which seems to be a general problem of highly mycotrophic plants with lacking or insufficient mycorrhizal colonization (see also Janos 2007).

Moreover, our basic assumption of CMN-mediated competition for nutrients between seedlings and large plants, can be accepted on base of the nutrient concentrations in seedling tissue and soil. The low seedling N:P ratios in those treatments, where large plant CMN-

connections were retained ('specific exclusion' and 'full CMN'; Fig. 5.12b), indicate that growth was rather N- than P-limited in these seedlings (Koerselman & Meuleman 1996), which resulted from CMN-mediated competition. This was confirmed by strong depletion of soil N, when large plant CMN-connections were retained, indicated by final soil N concentrations not exceeding the initial ones (Fig. 5.12a). In contrast, there was accumulation of N in soil of treatments without hyphal access by large plants ('CMN -all' and 'no CMN'). The stronger N-depletion in the 'full CMN'- and the 'specific exclusion'-treatments can only be explained by the retained large plant CMN-connections, as the respective seedling N contents were lower than in 'CMN -all' (Fig. 5.11c), where depletion was only intermediate (Fig. 5.12a). In this regard, it has to be considered that the extraordinarily low N-levels of ~ 0.1 mg kg soil $^{-1}$ (Marschner 2003) probably represent the remaining proportion of N that could not be depleted from the soil under the given conditions, thus probably reflecting a 'biological zero-level'. On base of this assumption, the lacking difference between initial and final N-levels suggests that the conjoint N demand of all CMN-connected host plants was exceeding the amount of applied fertilizer, thus causing the observed N-limitation. However, our third hypothesis that exclusion of highly mycotrophic large plants (*H. pilosella* and *P. lanceolata*) from the CMN would reduce the competitive pressure on seedlings, has to be rejected, as neither seedling N and P content, nor depletion of soil N and P were different within the 'specific exclusion' treatments. Even though the nutrient demands of large plants were probably species-specifically different due to different plant sizes and mycotrophy levels (Janos 2007; Hoeksema *et al.* 2010), differences in competitive pressure were not detected. The most likely explanation for these lacking differences is that even when disconnection of a highly competitive large plant from the CMN led to an increased availability of nutrients within the CMN-compartment, these nutrients were most likely rather transferred to the (remaining) three large plants than to the seedlings.

As mentioned above, our results indicate competition for N via CMN, which is in line with the study of Janouskova *et al.* (2011), who attributed seedling growth depressions in a nutrient-deficient substrate to N-depletion by an extraradical mycelium of neighboring large pants. However, in the present study, we were able to identify competition for N as being CMN-mediated, as the used approach allowed to partition N-transport via mass-flow and CMN by comparing 'CMN -all' and 'no CMN' values with the other treatments (Fig. 5.12a). Nevertheless, similar to Janouskova *et al.* (2011), we were not able to disentangle if seedling N-uptake was only reduced due to N-consumption by large plants or if fungal N consumption might also have played a role (Ocampo 1986; Moora & Zobel 1996, 1998; Janouskova 2011).

In this regard, we suggest that competition for N with AMF might have played a considerable role, as under N-deficiency, such as in our experiment, N is probably limiting both, plant and fungal growth (Johnson 2010). In this case, N acquired by a CMN would preferentially remain in fungal tissue instead of being transferred to host plants. This might also explain the N-limitation in 'CMN -all'-seedlings, which can otherwise neither be explained by large plant competition nor by insufficient soil N, since large plants were disconnected from the CMN and soil N was sufficient as indicated by N accumulation. However, irrespective of plant and fungal contributions, we could show that negative effects by retaining large plant CMN-connections were due to CMN-mediated competition for N. Our data clearly demonstrate, that under N-deficient conditions such as in the early successional stages of temperate open sand ecosystems, AMF are not only relevant for transfer of P, but can also play an important role in N-transfer to plants. This result contributes to the growing body of evidence that N-transfer may be important in arbuscular mycorrhizas (e.g., Subramanian & Charest 1999; Mäder *et al.* 2000; Govindarajulu *et al.* 2005).

Most interestingly, our results suggest that growth of highly mycotrophic seedlings may be limited to similar extents in both absence and presence of a CMN, but, however, that limitation may be shifted from P- to N-limitation. We conclude that large plants providing CMNs, are on the one hand, highly beneficial to strongly mycotrophic seedlings as they promote the 'activation' of P uptake. However, on the other hand, strong competitive large plant effects may completely neutralize any benefit, resulting in a net neutral CMN-effect. Even though this finding suggests a low relevance of CMNs for seedling establishment in terms of growth, we cannot rule out other beneficial effects due to CMN-promoted mycorrhization (Simard & Durall 2004), such as improved water status or defense against pathogens and herbivores (Gange & West 1994; Augé 2001; Sikes *et al.* 2009), which may increase seedling survival.

5.5.5 Implications of positive and negative CMN-effects for seedling establishment

Unexpectedly, net CMN-mediated seedling facilitation did not occur in the present study. Although neutral CMN-effects on seedling growth have been observed earlier (e.g., Grime *et al.* 1987; Moora & Zobel 1996; Kytoviita *et al.* 2003), the results of the present study are in contrast to a preliminary study, in which we observed clear net seedling facilitation by both a *H. pilosella* large plant monoculture and a large plant species mix of *H. pilosella* and *P. lanceolata* (Ch. 4). These discrepancies are most likely explained by different experimental designs, with CMN-connection of three instead of two large plants probably causing stronger

competitive effects and a consequently stronger compensation of the positive 'inoculation effect'. On the other hand, it is possible that a CMN, maintained by two *H. pilosella* large plants (Ch. 4) has a particularly high facilitation potential due to a favorable ratio between a strongly positive 'inoculation effect' and a relatively low competitive effect due to the low nutrient-demand of the relatively small *H. pilosella* plants. However, such a favorable constellation for seedling facilitation was not given in the present study. In contrast, the results of the present study indicate that the positive 'inoculation effect' due to enhanced P uptake and reduced C-costs for root colonization was completely overlaid by strong CMN-mediated competition with large plants and probably to some extent by increased CMN-costs to the seedling. As suggested in some recent studies, CMN-mediated competition for nutrients is driven by the relative C-supply of the connected host plants, with predominant transfer of CMN-nutrients to those plants that provide most C to the network (Lekberg *et al.* 2010; Hammer *et al.* 2011; Fellbaum 2012; Merrild *et al.* 2013; Weremijewicz & Janos 2013). Thus, the observed increased seedling C allocation to the CMN might also reflect a mechanism for acquiring higher amounts of CMN-nutrients. In this case, it is possible that the relatively low CMN-directed C allocation by the seedling - compared to the presumably much higher C allocation by large plants - was insufficient to enable a significant increase in uptake of CMN-nutrients. However, since quantitative assessment of total (intraradical and extraradical) mycorrhizal C-costs to the seedling was not possible, their relative importance for seedling growth limitations can hardly be estimated. Nevertheless, we found that CMN-provision did not only cause increased C-costs for CMN-maintenance but also reduced C-costs for root colonization, potentially compensating CMN-costs to some extent. Although in the present study net CMN-mediated facilitation of seedling growth by neighboring large plants was not observed, beneficial CMN-effects on seedling establishment on bare sand cannot be ruled out due to the clearly CMN-promoted root colonization and P uptake. However, we suggest that it is more likely that the observed negative effects were rather due restricted nutrient uptake as a result of nutrient competition by large plants. This is in line with several other studies, attributing net neutral or negative CMN-effects on seedling growth to competitive large plant effects (e.g., Eissenstat & Newman 1990; Kytoviita *et al.* 2003; Merrild *et al.* 2013). This is underlined by the finding that the only positive CMN-effect (Fig. 5.11a) was found in the ‘CMN -all’ treatment, where the beneficial 'inoculation effect' (Tab. 5.1) was not masked by competitive interactions via CMN.

5.6 Conclusions

The results of the present study showed that, although large plants may supply considerable amounts of C to a CMN, seedling CMN-costs may increase due to connection of large plants. Increased CMN-costs may thus represent a negative CMN-effect, counteracting positive CMN-effects by accelerated inoculation. For the first time we demonstrated that negative CMN-effects on seedlings may not exclusively be due to competition for CMN-nutrients, but may also be caused by increased mycorrhizal C-costs. Surprisingly, these CMN-costs were not related to the species identity and mycotrophic degree of the connected host plants, but rather with the extent of the CMN. Moreover, we conclude that CMN-growth might be particularly promoted by productive, highly mycotrophic plants, potentially acting as 'key species' for CMN-maintenance. Although not related to seedling facilitation, this might have important implications for P uptake in highly mycotrophic seedlings as provision of a CMN was clearly shown to accelerate seedling root colonization, thereby helping to overcome P-limitation, which seems to be a general problem of insufficiently colonized, highly mycotrophic plants, even under N-deficiency. Nevertheless, our study suggests that this generally positive 'inoculation effect' may be completely overlaid by strong negative CMN-effects due to CMN-mediated competition for N. We conclude that irrespective of the net CMN-effect on seedling growth, presence of competitors in a CMN may shift seedling P-limitation to N-limitation, thus decreasing the facilitative effects of the CMN. By disentangling the relevance of 'inoculation effects', seedling carbon drain and nutrient competition by large host plants in a CMN our study contributes to an improved understanding of the fundamental mechanisms driving mycorrhiza-induced seedling facilitation.

5.7 Authors contributions

I designed the experiment and accomplished the entire experimental work, data analysis, and writing of this chapter. Further contributions were given by S. Unger and W. Beyschlag, who assisted with data interpretation and writing. Further, I acknowledge C. Werner for making possible stable isotope analyses at BayCEER, E. Furlkröger, C. Schlüter and B. Teichner for support with plant cultivation and laboratory work.

CHAPTER 6
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Synthesis and Outlook

The main objective of this thesis was to disentangle mechanisms underlying root- and AMF-mediated foraging and unraveling the implications of their interplay for the competitive ability of a plant. The obtained results contribute to two major complexes of problems: (1) the question of the potential importance of AMF-mediated nutrient acquisition and competition for general plant performance and (2) the issue of the role of CMNs in plant-plant interactions.

6.1 Implications of AMF-mediated nutrient acquisition for plant performance in absence and presence of competition

Hoeksema *et al.* (2010) concluded from a meta-analysis on the 'context-dependency in plant response to inoculation with mycorrhizal fungi', that the ratio between root- and AMF-mediated contributions to a plant's total nutrient uptake is particularly dependent on plant species identity (see also Wang & Qiu 2006). The pronounced difference between the highly mycotrophic (coarse-rooted) forbs *Hieracium pilosella*, *Hypochaeris radicata* and *Plantago lanceolata* and the low mycotrophic (fine-rooted) grasses *Festuca psammophila* and *Corynephorus canescens* in the present study (Ch. 3) is a good example for completely contrasting foraging - and depletion - strategies of potential competitors. The performance of the coarse-rooted forbs was highly dependent on presence of AMF (Ch. 2, 3) as insufficient mycorrhizal root colonization lead to a general P limitation and restricted growth to a minimum, even when soil P was available in sufficient amounts (Ch. 3, 5), while in presence of mycorrhiza an adequate nutrition was reached. Moreover, strong C-investment into AMF revealed as a particularly efficient strategy for the rapid exploitation of bare soil patches (Ch. 3), providing potential competitive advantages. Thus, the results of Exp. 1 and 2 (Ch. 2, 3) clearly demonstrate the potentially high relevance of AMF for the foraging performance of grassland plants. Nevertheless, it may be difficult to make predictions on the competitive ability of a plant, based on AMF-mediated depletion as observed on the individual scale.

Although the results presented in Ch. 2 led to the conclusion that a highly mycotrophic life-style may be a very successful trait on the individual scale, it may be a disadvantageous

trait for the competitive ability as compared to a more root-mediated nutrition. The result that *H. pilosella* was clearly inferior to *P. lanceolata*, which exhibited a similarly high mycotrophy level, but allocated much higher proportions of carbon into root biomass, is in line with other studies reporting AMF-induced amplification of a competitive imbalance between plants of similar mycotrophy levels (e.g., Hetrick *et al.* 1994; Moora & Zobel 1996; Scheublin *et al.* 2007). In this case, size-symmetric root competition (Weiner 1986; Cahill & Casper 2000) is probably the decisive factor for the outcome of competition. This finding demonstrates the importance of considering both, mycotrophy level and root parameters of the involved species, when making predictions on the outcome of competition. The complex interplay between mycotrophy level and root system size certainly is one of the main causes for the lack of a consistent relation between mycotrophy level and competitive ability (e.g., Allen & Allen 1984; Hartnett *et al.* 1993; Smith *et al.* 1999; Scheublin *et al.* 2007; Daisog *et al.* 2012).

6.2 The role of CMNs in plant-plant interactions: mechanisms and implications

The emergence of CMNs between mycorrhizal plant individuals (Leake *et al.* 2004; van der Heijden & Horton 2009) and the associated loss of the exclusivity in the access to the proportion of nutrients taken up via AMF represents an important difference to (direct) root-mediated competition. In this regard, it has to be considered that neighboring mycorrhizal plants do not only compete for nutrients in the soil solution, but also for those nutrients that have already been taken up by a CMN (Newman *et al.* 1992). Recent work of Merrild *et al.* (2013) and Fellbaum *et al.* (2014) revealed that the amount of C allocated from a host plant to a CMN probably is the driving factor for the distribution of nutrients within the CMN, with a predominant nutrient transfer to hosts with high C input. Based on this, the authors hypothesize that competition for CMN-nutrients - in contrast to root-mediated competition - is size-asymmetric. With respect to the observed AMF-induced amplification of the competitive superiority of *P. lanceolata* over *H. pilosella* (Ch. 2), it has to be considered that *P. lanceolata* revealed as a 'key species' for CMN growth, indicating high C allocation to the CMN (Ch. 5, see also below). Thus, the competitive superiority of high productive, large-rooted plants such as *P. lanceolata* over less productive, small-rooted plants such as *H. pilosella* (see Ch. 2) might not only be due to advantages in size-symmetric root competition (Weiner 1986; Cahill & Casper 2000), but additionally, a result of asymmetric competition via CMN. The findings of the experiments presented in this thesis clearly emphasize the importance of considering CMNs, when trying to disentangle mycorrhizal effects on plant-plant interactions. In this

regard, CMNs deserve particular attention, since, besides competitive CMN-mediated interactions, there may also be positive, i.e. facilitative, CMN-mediated interactions between neighboring mycorrhizal plants.

The results of Exp. 3 and 4 (Ch. 4, 5)led to the conclusion that accelerated mycorrhizal colonization of seedling roots due to connection to a CMN probably is the most important mechanism underlying CMN-mediated seedling facilitation (see also Leake *et al.* 2004; Simard & Durall 2004). Moreover, we concluded that this 'inoculation effect' is of particular relevance for the establishment of seedlings belonging to highly mycotrophic species such as e.g. *H. pilosella* and *P. lanceolata*. As mentioned above, seedling establishment in these species is severely P-limited under lacking (Ch. 3) or insufficient (Ch. 5) mycorrhizal root colonization - thus, the CMN-mediated 'inoculation effect' is of great importance for these plant species. Since a quantity of grassland species is highly mycotrophic (Wang & Qiu 2006), CMNs can be expected to be of great ecological significance and certainly play an important role in grassland systems. From the results of Exp. 3 (Ch. 4) it becomes clear that the net facilitation potential of a CMN is dependent on the species identity of the adult plants, which can be attributed to simultaneous competitive pressure, overlaying positive effects. Here, pronounced mycelium growth, combined with a generally low competitive pressure, as in the small-rooted and almost obligately mycotrophic *H. pilosella*, seems to be most favorable for efficient CMN-mediated seedling facilitation. The finding that CMN-mediated seedling facilitation by adult plants is not restricted to seedlings of the own species (Ch. 4), should have important implications for plant-plant interactions on the community level and transition dynamics between subsequent successional stages.

Contradictory to our expectation, the ^{13}C-labeling approach (Exp. 4, Ch. 5) revealed that CMN-C-costs to seedlings rather increased than decreased because of an increased CMN-size due to the connection to neighboring adult plants. Thus, the 'inoculation effect' might be the only positive CMN-mediated effect of adult plants on seedlings, counteracted by both, increased CMN-costs to seedlings and competitive pressure, exerted by neighboring adults. Indeed, CMN-mediated net facilitative effects usually occur only temporary and diminish due to increasing nutrient demand by seedlings, finally resulting in net competition (Leake *et al.* 2004; Beltrán *et al.* 2012). Accordingly, Exp. 4 (Ch. 5) revealed that provision of a CMN by neighboring adult plants was highly important for initiation of seedling growth (facilitative effect), while ongoing connection of adult plants induced a strict suppression of seedling growth (competitive effect), thus resulting in a net neutral effect. Although each of the two counteracting effects was resource-mediated, facilitation was P-mediated whereas competition

was N-mediated. This could be attributed to the species-specific high mycotrophy level of the seedlings and the low availability of soil N, respectively. Thus both, the mycotrophy level as well as the nutritional soil conditions do play an essential role in the net CMN-mediated adult plant effect on seedlings.

As a final conclusion it can be stated that besides root properties, the species-specific mycotrophy level of a plant is of major importance for belowground interactions with other plants. Further, it became clear that the mere capacity of a mycorrhizal plant to deplete nutrients in absence of competition is an unsuitable indicator for its competitive ability. For a full understanding of net plant-plant interactions, consideration of (positive and negative) CMN-mediated interactions and their interplay with (negative) root-mediated interactions is essential. Finally, all these parameters must always be interpreted in relation to the respective nutritional soil conditions.

6.3 Outlook

The present study revealed some novel insights into some potentially important mechanisms underlying the mediation of plant-plant interactions by AMF. However, due to the limited number of experimental species, further experiments using an extended spectrum of plant and fungal species are required for generalization of these results. Further, mesocosm and field studies will be necessary for validation of the obtained 'laboratory results' for natural conditions, as variances in the environmental conditions may have considerable impact on AMF-effects on plant-plant interactions (Smith & Read 2008). Due to their enormous complexity, particular attention should be given to the - evidently highly significant - role of CMNs, because knowledge about the function of these highly complex structures in the context of plant-plant interactions is still very poor (see e.g., Merrild *et al.* 2013). Experimental systems similar to that used in Exp. 4 (Ch. 5), enabling specific exclusion of plant individuals from a CMN, may provide a suitable approach for this.

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INDEX OF FIGURES AND TABLES

PUBLICATIONS & CONFERENCE CONTRIBUTIONS

Publications

2014 Höpfner, Friede, Unger and Beyschlag: Potential advantages of highly mycotrophic foraging for the establishment of early successional pioneer plants on sand. Functional Plant Biology. http://dx.doi.org/10.1071/FP14097.

Conference contributions

2013 Höpfner, Friede, Unger and Beyschlag: Carbon allocation trade-off between arbuscular mycorrhizal fungi and roots reveals contrasting foraging strategies in pioneer plant species on sand. Oral and poster presentation, Deutsche Botanikertagung, Oct 2013.

2013 Friede, Höpfner, Unger and Beyschlag: Contrasting foraging strategies of co-existing grassland plant species: The relevance of mycotrophy. Oral presentation, 43rd Annual Conference of the GfÖ, Sept 2013.

Höpfner and Beyschlag: Presence of arbuscular mycorrhiza shifts interspecific competition towards facilitation at low P availability. Oral presentation, 43rd Annual Conference of the GfÖ, Sept 2013.

Höpfner and Beyschlag: Obligate mycotrophy – an expensive strategy under competitive pressure? Poster presentation, GfÖ AK Experimental Ecology Meeting, Mar 2013.

2011 Höpfner, Hefner, Werner and Beyschlag: Effects of arbuscular mycorrhiza on competition and facilitation between plants. Oral presentation, 41st Annual Conference of the GfÖ, Sept 2011.

2010 Höpfner and Beyschlag: Response of terrestrial cyanobacteria to rising CO2 and desiccation. Poster presentation, GfÖ AK Experimental Ecology Meeting, Mar 2010.

Awards

2013 Poster award of the German Botanical Society at the „Botanikertagung“, Oct 2013, Tübingen.

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ERKLÄRUNG

Hiermit versichere ich, dass ich die vorliegende Dissertation selbständig erarbeitet habe und alle Quellen, Hilfsmittel und Beiträge anderer Mitarbeiter angegeben sind.

Weiterhin erkläre ich, dass die vorliegende Dissertation weder vollständig noch teilweise einer anderen Fakultät mit dem Ziel vorgelegt worden ist, einen akademischen Titel zu erwerben und dass dies mein erster Promotionsversuch ist.

Bielefeld, 12. Dezember 2014